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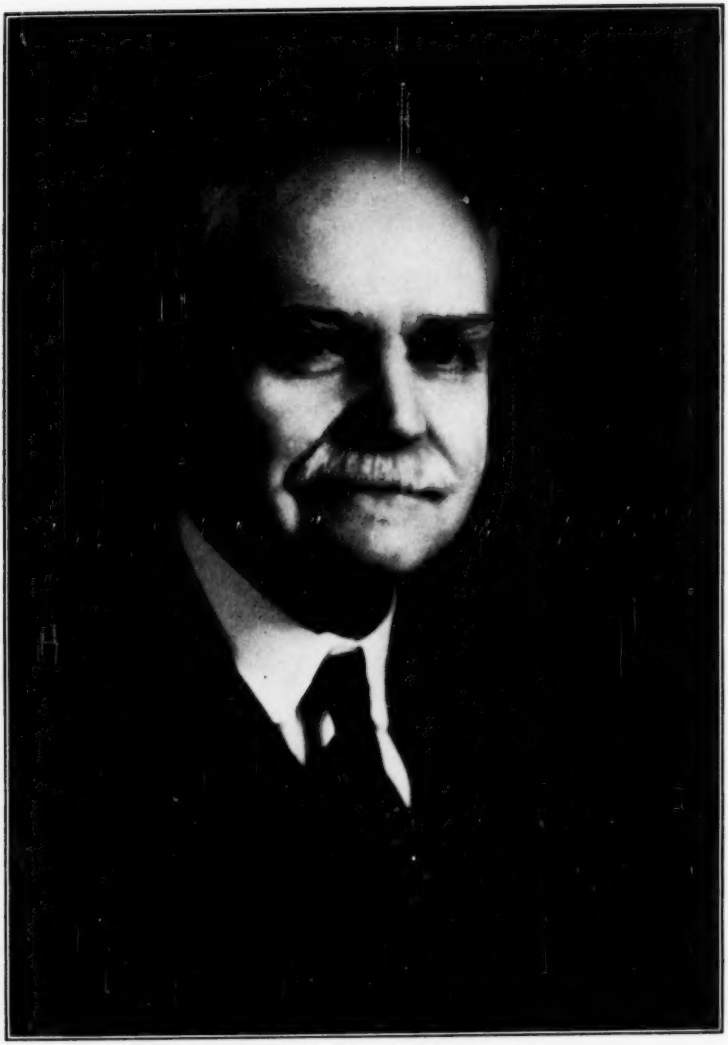

William Henry Long, 1867–1947

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WILLIAM HENRY LONG, 1867–1947

JOHN A. STEVENSON

William Henry Long was born March 7, 1867, in Navarro Co., Texas, and died December 10, 1947 at Albuquerque, New Mexico, where he had long resided. He took his undergraduate work at Baylor University, Waco, Texas, receiving the A.B. degree in 1888. Following graduation he served as Professor of Natural Sciences with his Alma Mater until 1892 when he assumed a similar position at Burleson College, Greenville, Texas.

Feeling the need for further botanical training he severed his connection with this institution in 1899 to take graduate work in his chosen field at the University of Texas, under the guidance of Professors Wm. L. Bray and W. M. Wheeler, where he also served as assistant in botany for the period 1900–1901. As a result of his studies here the A.M. degree was given him in 1900. His first mycological paper on the distribution of the fungi of Austin and vicinity was prepared and published at this time. For the next nine years he was Professor of Botany at North Texas State Normal College at Denton. Here with increased tempo he continued his mycological studies with emphasis on the Uredinales. During this time he published his classical paper on the Ravenelias of the United States and Mexico as well as several on other rusts and on the Phalloideae of Texas.

Throughout his career Long was an avid collector of fungi, and very early in his scientific work commenced assembling an herbarium both by personal collecting and by exchange. Unfor-

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tunately, most of his Texas specimens were lost in a fire in his home at Denton about 1907. Many of his collections, however, had been shared with other mycologists so that the loss was not as serious as it would have been had it not been for this life-long habit of collecting abundantly and exchanging freely.

During this time, in addition to extensive mycological field work in the vicinity of Denton, several summers were spent at Cornell University in similar activities under the direction of Professor Geo. F. Atkinson. These studies were ultimately the basis of his doctoral dissertation presented in 1917 to the University of Texas which granted the Ph.D. degree.

Long entered federal service in January, 1910 as a member of the editorial staff of the Experiment Station Record in the Department of Agriculture at Washington, being assigned to the field of "agricultural botany, bacteriology and vegetable pathology." This sedentary existence was not particularly to his liking and after some 18 months he transferred to the then comparatively new Office of Forest Pathology in the Bureau of Plant Industry. Here under the leadership of Haven Metcalf he became one of the group, which included George G. Hedgcock, P. Spaulding, E. P. Meinecke, J. R. Weir and others, who pioneered in the development of forest disease work in the United States.

Following a short stay at Washington headquarters where he studied wood rots, and forest-tree rusts, particularly those of the genus *Gymnosporangium*, he was assigned to head up the work in the Southwestern States with headquarters at Albuquerque, New Mexico. Here he spent the remainder of his career, for a time covering a territory which extended from Arizona to Florida, but soon restricting his activities to Arizona, New Mexico, and Colorado. Some forty technical papers cover his work for the period, ranging over the many problems encountered in the forests of his region, all studied in close cooperation with the U. S. Forest Service.

In addition to continued work on forest-tree rusts and wood rots in general, his outstanding accomplishments were the development of brush disposal methods on national forests, initiation of sylvicultural methods of handling yellow pine stands infested by

mistletoe, and studies on the fungi which rot railroad ties, fence posts, and poles, aimed at preventing their attack.

Following his retirement in July, 1937, he took up with great enthusiasm what had been one of his mycological hobbies for many years, a study of the taxonomy of the Gasteromycetes. In this work he gave particular attention to those of the Southwest and with the assistance of David J. Stouffer and other experienced collectors, brought together a very extensive and valuable series of these fungi. The results of Long's studies are for the most part in the series of papers under the general title, Studies in the Gasteromycetes. His private herbarium was bequeathed to the Smithsonian Institution.

Dr. Long was a member of several technical organizations during his active career, among which were the Mycological Society of America, the American Phytopathological Society, the Texas Academy of Science, the Ecological Society of America, the Botanical Society of Washington.

There follows a selected list of his writings in Mycology and Phytopathology. Papers in other fields, as well as abstracts and popular articles, have been omitted.

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AN ENDOCOCHLUS HAVING BINARY HELICOID THALLI OF LEFT-HANDED ROTATION

CHARLES DRECHSLER [1]

(WITH 6 FIGURES)

Several maizemeal-agar plate cultures which after having been overgrown with mycelium of *Pythium mamillatum* Meurs had been further planted on January 3, 1947, with small quantities of leaf mold kindly collected by Dr. E. B. Toole in an oak wood near Greensboro, North Carolina, in December, 1946, showed, on microscopical examination twenty-seven days later, sparse stands of erect beaked conidia arising in scattered areas from prostrate hyphae often arranged more or less radially. The general aspect of the conidial apparatus indicated clearly a species of *Endocochlus*. While the robust stature of the erect spores was rather strongly suggestive of my *E. gigas* (**6**: 368–371), their longer and accordingly much more conspicuous empty apical beaks made for an appearance alien to that species as well as to the smaller congeneric forms I have described under the binomials *E. asteroides* (**5**: 8–15) and *E. brachysporus* (**6**: 364–367). Closer examination, in which attention was given also to the vegetative and the sexual reproductive stages associated with the conidial apparatus, brought to light morphological peculiarities amply distinguishing the fungus here concerned as a new member of the genus.

Like the congeneric forms previously described, the new species subsists through parasitism on a relatively large terricolous *Amoeba*. Animals in the earlier stages of attack (FIG. 1, *A*; FIG. 2, *A*; FIG. 3, *A*; FIG. 4, *A*) were commonly found to measure from 100 to 130 μ in width when drawn into a rounded shape. They

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were enveloped individually by a firm pellicle that for the most part was cast into broadly undulating folds. Their hyaline, obscurely granular protoplasm surrounded a single prolate ellipsoidal nucleus (FIG. 1, *A*, *n*; FIG. 2, *A*, *n*; FIG. 3, *A*, *n*; FIG. 4, *A*, *n*), commonly 27 to 32 μ long and 18 to 21 μ wide, that contained at its periphery a large number of globose bodies about 1 μ in diameter. At the two poles of the nucleus these bodies, presumably consisting of chromatin material, were massed several layers deep, while in the equatorial region they were present usually in a single layer. Owing to the deeper accumulation of globose bodies at the poles the homogeneous central portion of the nucleus was often approximately equal in length and width. Many infected animals, as also many healthy individuals of the same species, contained one or more specimens of the testaceous rhizopod *Euglypha levis* (Ehrenb.) Perty (FIG. 1, *A*, *w*; FIG. 2, *A*, *w*), which, being abundant in the cultures, had apparently been ingested to serve as food material.

Because of close resemblances in nuclear organization, the protozoan here in question is held referable to *Amoeba papyracea* Penard (8: 201). While the measurements given by Penard for width of rounded individuals—minimum 176 μ, maximum 198 μ—are considerably in excess of those prevailing in my cultures, smaller dimensions would seem to be more or less normal in *Amoeba* populations developing on agar substrata. Previous to its appearance as the animal host of the new *Endocochlus* the protozoan had not been noted in the many agar plate cultures prepared from leaf mold from different sources over a period of more than ten years. Whether its failure to develop more often in plate cultures is due to limited distribution or to special requirements for multiplication, its absence in any material naturally precludes development of the zoöpagaceous form habitually subsisting on it.

Encounter between specimens of *Amoeba papyracea* and the fungus takes place much as in the several other species of *Endocochlus* made known earlier. When the *Amoeba* in its ordinary locomotion comes in contact with a conidium of the parasite the conidium remains affixed to the animal's pellicle, apparently through adhesion. From the adhering region the spore soon puts forth a bulbous protuberance, about 3 or 4 μ wide, which serves presumably as an ap-

pressorium since its rather thick wall usually shows later the yellowish coloration characteristic of adhesive structures in the Zoöpagaceae. The protuberance soon begins to elongate apically as a germ tube, in many instances at first pushing the pellicle inward to form a funnel-shaped depression. After the pellicle has been ruptured under increasing pressure the germ tube continues its growth some little distance into the protoplasmic interior. Elongation then ceases, and the narrowed tip of the germ tube abruptly begins expanding into a bulbous swelling (FIG. 1, *A*, *a*). Through progressive vacuolization the contents of the adhering conidium migrate gradually into the terminal swelling. When this migration is completed the externally adhering conidial envelope is wholly empty, as is also the germ tube on whose tip is then borne a prolate ellipsoidal or nuciform cell (FIG. 1, *A*, *b*) densely filled with protoplasm. Disturbances deriving from the movements of the animal readily suffice to detach the nuciform cell from its slender support. Following such disjunction the empty germ tube together with the conidial envelope is shed by the animal; while simultaneously the nuciform cell, or infective body (FIG. 1, *A*, *c*), immersed in its mobile protoplasmic ambient, begins autonomous parasitic development.

With the assimilation of nourishment from the host protoplasm, the infective body here sometimes grows out nearly as in the other known species of *Endocochlus*, by putting forth from its distal end a single prolongation that usually begins curving at an early stage (FIG. 1, *A*, *d*, *e*; FIG. 2, *A*, *a*; FIG. 3, *A*, *a-c*) to form a close helical coil (FIG. 1, *A*, *f*; FIG. 3, *A*, *d*, *e*), which, after describing one and one-half to two turns, may branch dichotomously (FIG. 3, *A*, *f*, *g*). Even where only a single thallodic coil is formed the present fungus shows for some time, if not to the end, a distinctive peculiarity in that the prolongation is, as a rule, at least in its proximal portion, conspicuously narrower than the infective body, so that this body stands out as a proximal swelling. In contrast, the infective bodies of congeneric species, and also of those species of *Cochlonema* having a similar manner of invasion, grow out distally at an increasing width and thus become merged indistinguishably with the resulting thallus.

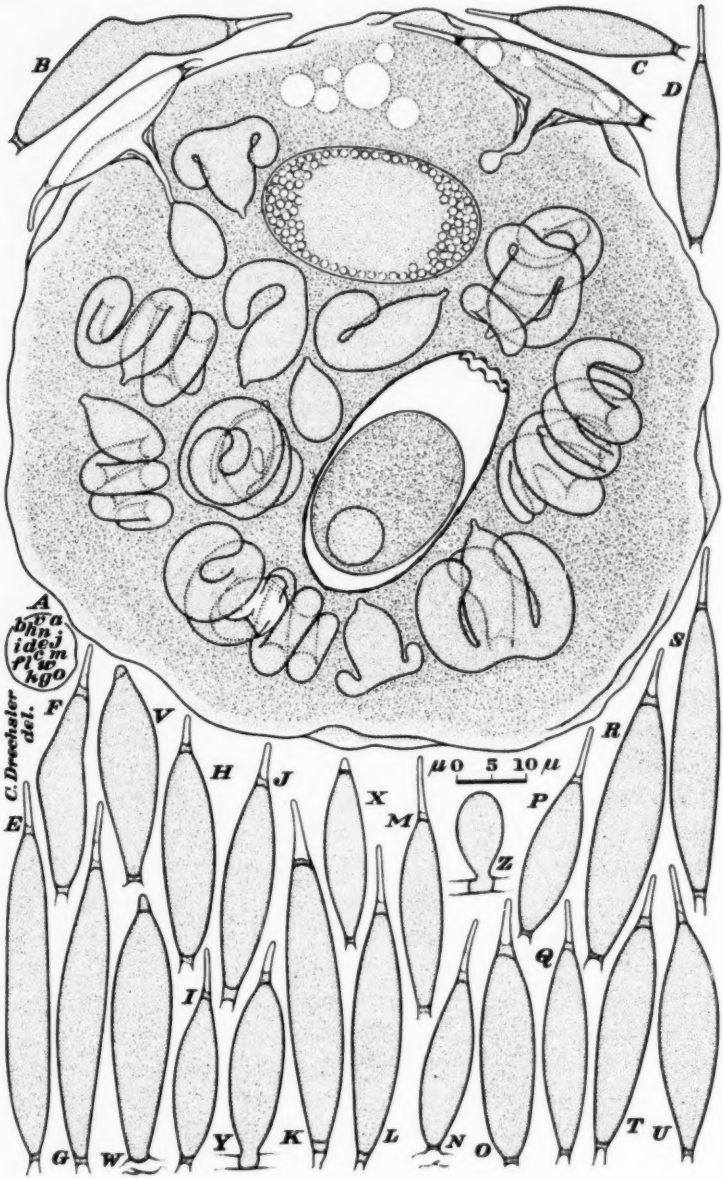

FIG. 1. *Endocochlus binarius.*

Although the production of a single thallodic coil is hardly infrequent in the present fungus, the much more usual manner of development here is by the extension of two thallodic coils. The two coils arise as small buds from opposite sides at the distal end of the infective body (FIG. 1, *A, g*). Through continued elongation, together usually with some distal widening and with constantly winding curvature (FIG. 1, *A, h*), they develop, as a rule, on opposite sides of the infective body, into helical coils which commonly remain unbranched until they have described one and one-half to two turns (FIG. 1, *A, i-l;* FIG. 2, *A, b-h;* FIG. 3, *A, h, i;* FIG. 4, *A, a, b*). Somewhat contrary to expectations that might be entertained, the coils are not oriented usually with their axes extending away directly from the sides of the infective body to which, respectively, they are attached, but instead are oriented with their axes perpendicular to the plane passing lengthwise through the middle of the infective cell and bisecting the basal attachments of both coils. The considerable measure of uniformity in positional relationships of the binary thallodic coils—a measure of uniformity seeming all the more remarkable because it is achieved despite constant disturbance from promiscuous movement of the protoplasmic ambient—is to be attributed to unusually consistent directive tendencies in growth that become manifest early in the elongation of the buds. After the initial abrupt curvature of the buds toward the proximal end of the infective body (FIG. 1, *A, g*), further curvature is such that if the infective body lies with its longitudinal axis in the plane of the microscopical field and with its proximal end directed toward the observer, the bud arising from the right side of the distal end will elongate with its advancing tip interposed between the observer and the infective body, while the bud from the left side of the distal end will elongate with its advancing tip passing underneath the infective body into partial concealment from the observer (FIG. 1, *A, h*). The three-dimensional curvature thus initiated is continued with further growth of the two buds; so that with the infective body oriented in the way just described, the bud arising on the right side of the distal end is soon found prolonged into a helical coil overlying the infective body and with its axis directed toward the observer in a line nearly parallel with his line of vision, while the bud arising from the left side of the distal end

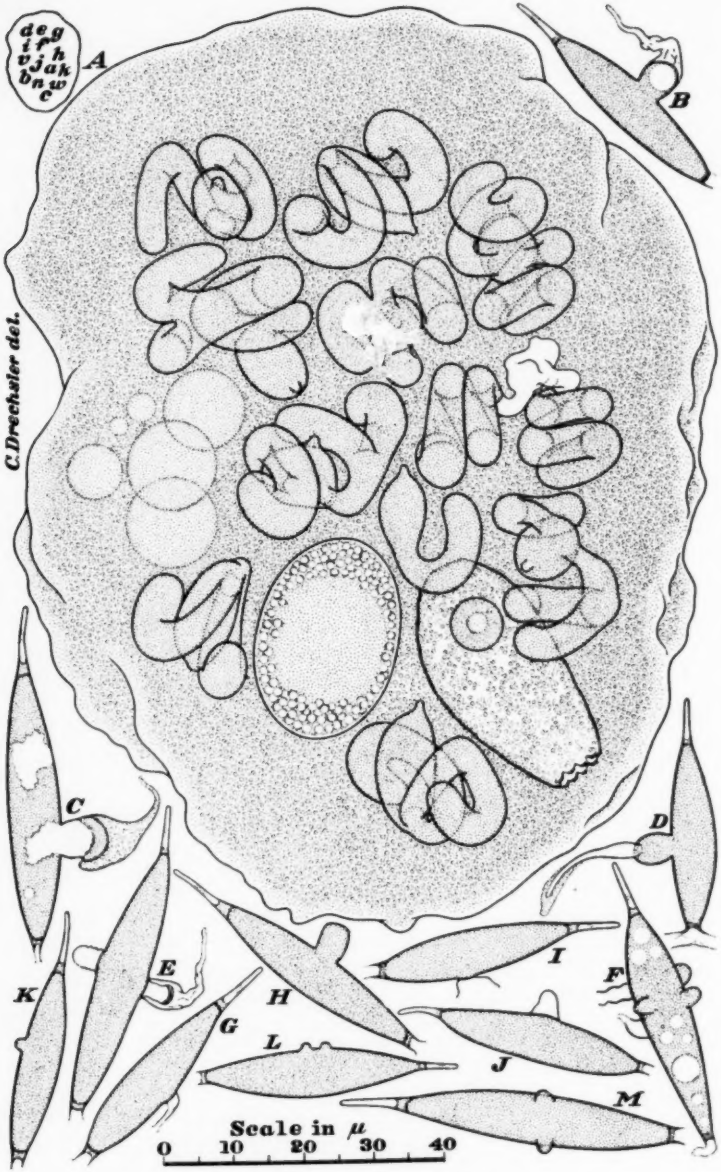

FIG. 2. *Endocochlus binarius.*

will be found extended into a similar coil underneath the infective body and with its axis directed vertically downward in a line likewise approximately parallel to the line of vision (FIG. 1, *A, l;* FIG. 3, *A, h;* FIG. 4, *A, b*). Curvature in similar relationship to the infective body and in the same direction of rotation prevails also where only one thallodic coil is produced, as well as in the occasional instances where three coils are formed (FIG. 1, *A, o*). Thus, regardless of variation in number, the coils consistently show rotation of the same direction as is found in the threads of a left-handed screw. To cite familiar examples from phanerogamic plants, the rotation shown here corresponds to that displayed in the twining stems of the common hop, *Humulus lupulus* L., and the black bindweed, *Polygonum convolvulus* L. Among fungi similar left-handed rotation occurs as a distinctive feature in the helicoid ascospores of my *Cochliobolus heterostrophus* (**2, 4**), in the conidia of my *Harposporium helicoides* (**7**), in the coiled sporogenous tips of the sporophores of my *Helicocephalum oligosporum* (**3**), as well as in the aerial sporogenous branches and spore chains of some helicoid species of *Streptomyces* (= *Actinomyces*) as, for example, *S. lavendulae* (Waksm. & Curt.) Waksm. & Henr. (**1**: 150–151; **9**; **10**: 944).

Since both of the two thallodic coils usually produced show rotation in the same direction they could well be regarded, especially during their earlier stages of growth, as making up a single binary helicoid structure modified midway between the ends by the presence of the protuberant infective cell. In many instances, however, the continuity of the combined structure is interrupted through evacuation of the infective body together usually with a proximal portion of the thallodic coils (FIG. 1, *A, k;* FIG. 2, *A, h;* FIG. 4, *A, a*); the larger, living portion of each coil then becoming delimited proximally by a retaining wall. The empty envelopes of infective bodies, after some time, often vanish completely from sight; so that, eventually, many of the larger thallodic coils (FIG. 3, *A, j;* FIG. 4, *A, c*) come to appear little different, at the proximal end, from those of other species of *Endocochlus*, where the infective body, because of its broad elongation from the full width of its distal end, merges indistinguishably with the thallus.

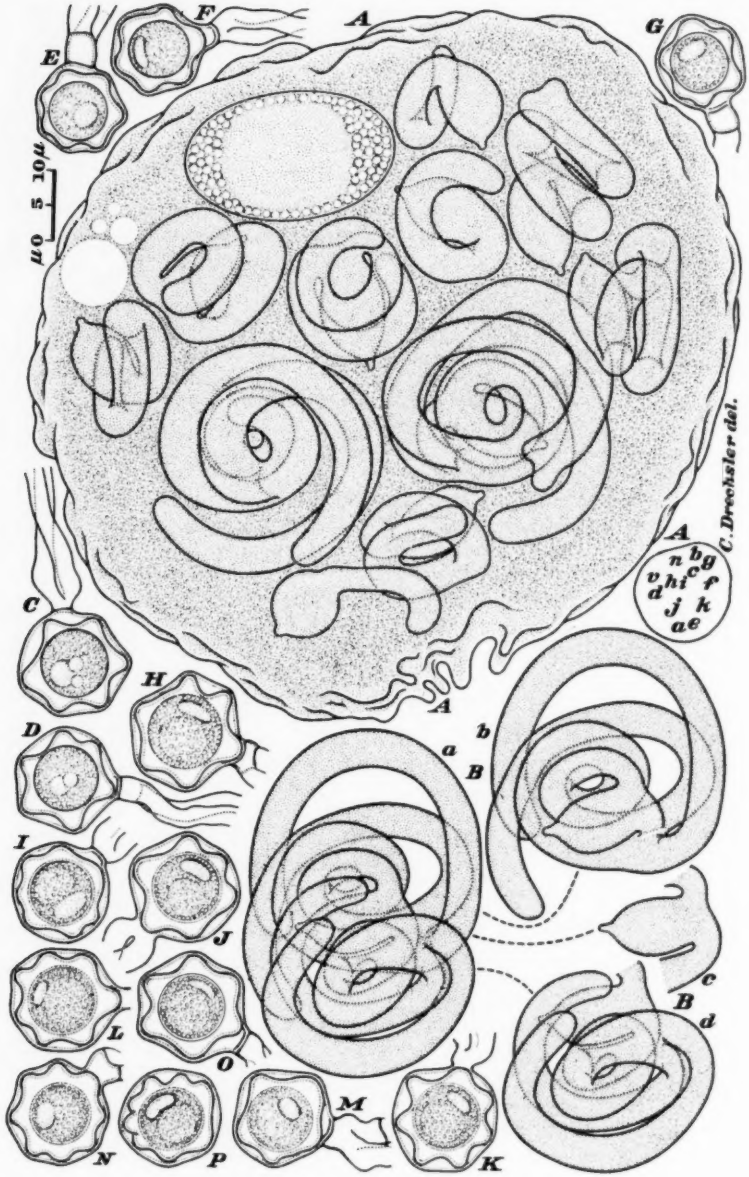

FIG. 3. *Endocochlus binarius.*

The larger thallodic coils are branched in varying measure, as many as three successive bifurcations often being recognizable (FIG. 4, *A, d*). Whether a coil is of solitary (FIG. 2, *A, i;* FIG. 3, *A, f, g*) or of twinned (FIG. 1, *A, m.* FIG. 3, *A, j, k; B, a-d.* FIG. 4, *A, c, d*) origin, its first dichotomy usually takes place after one and one-half to two turns have been formed, though thalli are not rare wherein much earlier bifurcation is clearly evident (FIG. 2, *A, j, k*). Following the first dichotomy, elongation of the branches is marked by diminished curvature, with the result that a thallodic coil whose first two turns may have an outer diameter equal to the length of the parent infective cell will after three successive dichotomies extend its eight branches rangily over an orbit three or four times wider (FIG. 4, *A, d*). Owing apparently to their rather open arrangement the longer terminal elements of well developed thalli are somewhat readily displaced from their original positions when they are jostled by other thalli in an animal under multiple attack. Accordingly in later stages of infection the larger thalli lose much of their geometrical symmetry as they are pressed together closer and closer. Before the protozoan host is incapacitated for further movement, primarily from advanced depletion of its protoplasm, the several thalli become confusedly intertangled in a dense clew (FIG. 5, *A, B*).

Although for a time after its disablement the infected specimen of *Amoeba papyracea* continues to operate its contractile vacuole (FIG. 5, *A, v; B, v*), further expropriation of its protoplasm and progressive degeneration of its nucleus (FIG. 5, *A, n; B, n*) eventually leads to the extinction of all signs of life. At this stage, or frequently a little earlier, the several thalli begin reproductive development. Where an infective cell is still present it often serves directly in putting forth one (FIG. 6, *A, a*) or more (FIG. 6, *B, a, b*) reproductive hyphae, while others are being extended from proximal positions on the associated coil (FIG. 6, *A, b*) or coils (FIG. 6, *B, c, d*). Naturally in those instances where the infective cell has been lost, all reproductive hyphae (FIG. 6, *C, a-h*) arise from the thallus, and in some instances all reproductive hyphae (FIG. 6, *D, a-g*) originate from the thallodic coils even where the infective body remains intact. The growth of the reproductive hyphae (FIG. 6, *E, a-d*) is accompanied by vacuolization and evacuation of

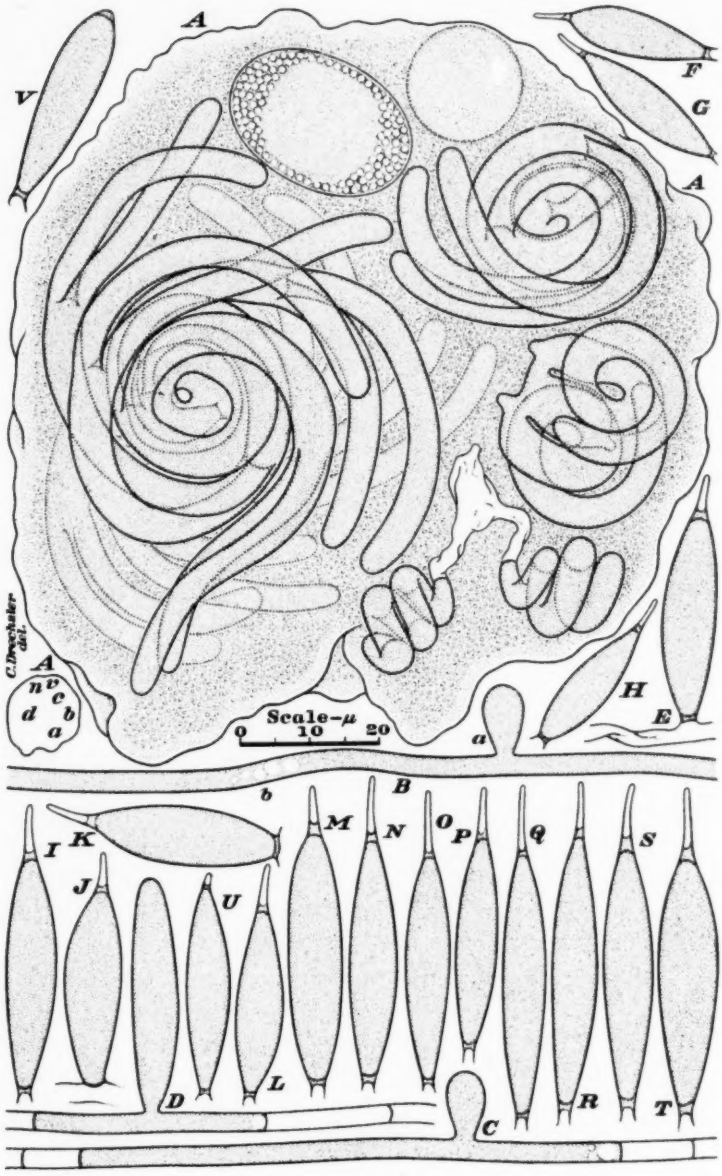

FIG. 4. *Endocochlus binarius.*

the thallus; the loss of contents usually becoming noticeable first in the distal parts of the coil (FIG. 6, *A*, *B*) and later in more proximal parts. Retaining walls are laid down at intervals in the progressive withdrawal of protoplasm (FIG. 6, *E*), much as in the other species of *Endocochlus*, as well as in most species of *Cochlonema*.

The reproductive hyphae destined to serve in the development of conidia push their way to the animal's integument without establishing any definite positional relationships to one another. On breaking through the pellicle they elongate radially from the dead animal until they extend procumbently over the surface of the culture for distances frequently of 2.5 to 3 mm. They usually show only rather gently curving deviations from their generally straightforward courses, and only small departures from their usual width of about 3 μ. After reaching their definitive length they soon put forth erect protuberances (FIG. 4, *B*, *a*; FIG. 5, *C*, *a-c*) often at intervals of 75 to 125 μ. Thereupon the hyphal contents near the middle of each interval become noticeably vacuolate (FIG. 4, *B*, *b*). As material is supplied for further growth of the protuberances the vacuolate portions are emptied and retaining walls are formed. Continued withdrawal of protoplasm soon leads to evacuation of an adjacent hyphal portion and deposition of another wall closer to the growing protuberance (FIG. 4, *C*. FIG. 5, *D*, *a-c*; *E*). With repetition of the process the living hyphal segment is further reduced (FIG. 4, *D*. FIG. 5, *F*, *a*; *G*, *a*), and finally, as a rule, the remaining hyphal contents migrate into the erect outgrowth, which then is delimited a little above the base by a thickish septum (FIG. 4, *E*. FIG. 5, *F*, *b*; *G*, *b*). During the later stages of its growth, the erect body usually extends upward a narrow apical prolongation, which is soon delimited at the base by a rather thick wall or somewhat irregular plug-like partition.

Although the erect conidium thus formed resembles that of *Endocochlus gigas* in the fusoid shape of the living cell, it is of generally greater dimensions throughout (FIG. 1, *B-Z*. FIG. 4, *F-V*. FIG. 5, *H*, *a-z*; *I*, *a-z*; *J*, *a-z*). It is distinguished more especially, however, by its narrow apical appendage, which as a rule is not only decidedly longer in actual measurement than the corresponding part in *E. gigas*, but is markedly longer also in comparison with the living cell; though in occasional specimens the appendage may be small

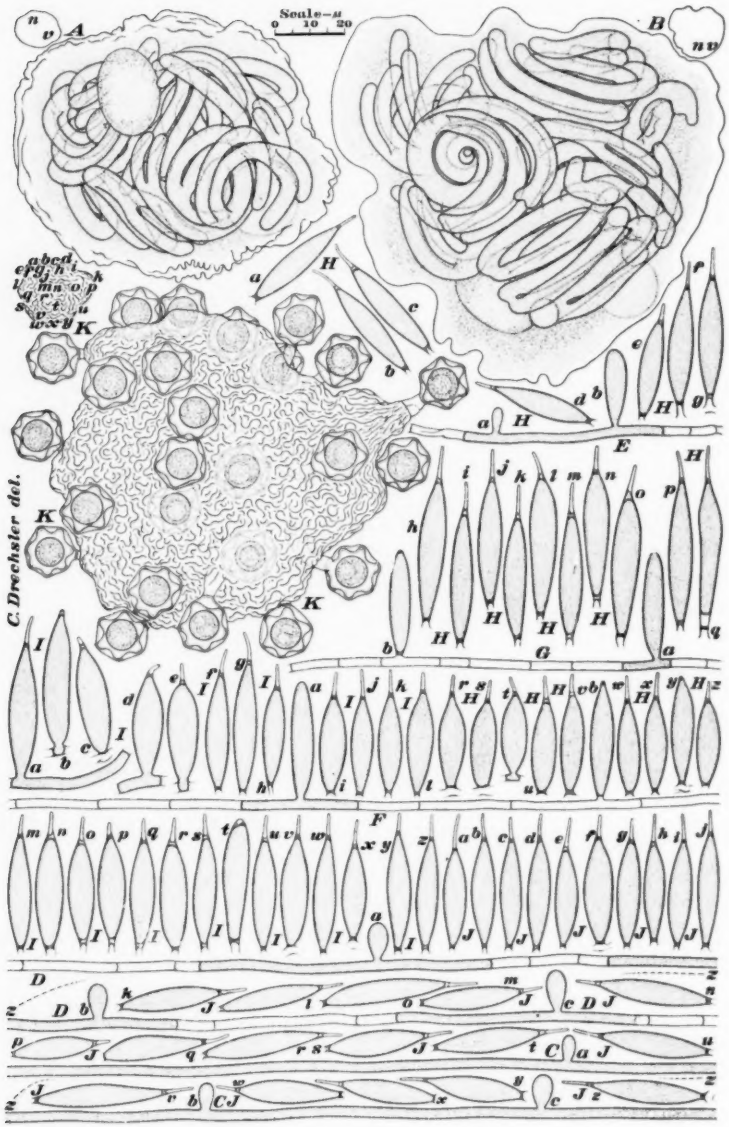

FIG. 5. *Endocochlus binarius.*

(FIG. 1, *X*; FIG. 4, *U*; FIG. 5, *I*, *c*, *t*), or may be present merely as a minute cap of solid wall material (FIG. 1, *V*, *W*. FIG. 4, *V*. FIG. 5, *F*, *b*; *G*, *b*; *H*, *y*; *I*, *b*), or in very imperfect specimens may be wholly absent (FIG. 1, *Z*). When the mature conidium becomes detached, it does not usually separate from the empty hypha flush with the lower side of its basal wall but is abjointed commonly at a distance of 0.5 to 2 μ or more rarely as much as 3 μ, from this wall; so that the spore carries at its base a curious membranous prolongation, a little like an appendage. This somewhat untidy manner of separation is not governed by chance, for the portion of hyphal membrane that accompanies the spore is always noticeably thicker than the hyphal envelope generally, and thus must have undergone earlier some special modification. In some instances where the basal septum was formed flush with the parent hypha, the membranous prolongation may include a cylindrical portion of the hyphal envelope (FIG. 1, *N*, *W*. FIG. 4, *J*. FIG. 5, *H*, *r*, *s*, *y*; *I*, *c*, *v*, *x*; *J*, *f*, *z*). Again, in occasional instances where because of incomplete transfer of protoplasmic contents the living cell of the detached spore includes a portion of the parent hypha (FIG. 1, *Y*, *Z*. FIG. 5, *H*, *t*; *I*, *a*, *b*, *d*, *e*), a short cylindrical portion of empty hyphal envelope is present at each of the two basal ends.

Germination has been observed taking place only in the conidia that have become affixed to the pellicle of a host animal. Nevertheless, in cultures where the fungus has been active on a large scale for a few days, the conidia strewn about on the substratum usually include many individuals with lateral outgrowths and membranous attachments of various shapes (FIG. 2, *B-M*). These modifications clearly derive from germinative development frustrated at different stages. In resisting infection from individual conidia of the fungus, *Amoeba papyracea* seems much more often successful than any other rhizopod I have hitherto seen undergoing attack by any zoöpagaceous parasite. In several observed instances the animal protruded a pseudopodium at the place of attack, thereby eventually confining the germ hypha in a small narrow-necked pouch. By persistent rotational movement of the animal the slender attachment was twisted off, and the pouch, consisting of a piece of pellicle and some protoplasm, was left behind adhering to the conidium (FIG. 2, *C*). The host animal appeared to carry out

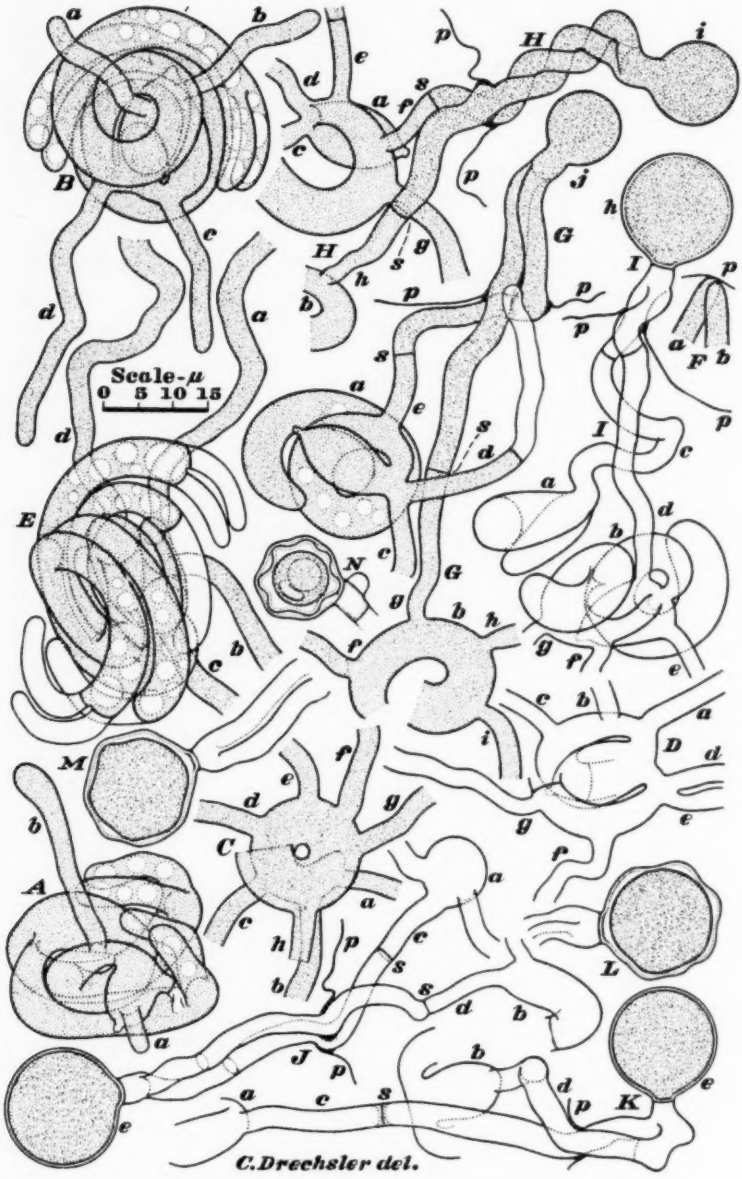

FIG. 6. *Endocochlus binarius.*

such defensive maneuvers repeatedly with different conidia, even when it was already infected with many growing thalli and thus was assuredly doomed. The conidia, on their part, were by no means rendered harmless by being sloughed off. If they had produced a long germ tube in their unsuccessful venture, the protoplasma in the tube was withdrawn backward into the spore envelope itself (FIG. 2, *E-G*), or into a short protruding stump (FIG. 2, *B, D, H*). If only appressoria had been formed, they were likewise evacuated when of large size (FIG. 2, *J*), but when relatively small (FIG. 2, *K-M*) were often left unchanged. Some conidia bore four or five frustrated germinative structures—appressoria and evacuated germ hyphae (FIG. 2, *F*)—thereby giving evidence of a corresponding number of unsuccessful attempts at infection.

The development of zygospores is initiated by reproductive hyphae of about the same width as those concerned in the formation of conidia. These hyphae make known their distinctive function by coming together in pairs inside the animal—the two members of a pair (FIG. 6, *F, a, b*) directing their growth in such wise as to bring their tips in contact with each other and at the same time with the inner surface of the animal's pellicle (FIG. 6, *F, p*). Pairing apparently never takes place between hyphae arising from the same vegetative thallus, single or binary; for in all instances where the difficulties of observation occasioned by the intertangling of fungus coils are not too troublesome, the two members of a pair are always traceable to separate thalli. Thus, in the reproductive apparatus shown in figure 6, *G*, where the two thalli *a* and *b* have each produced several reproductive hyphae, no pairing took place either between the three hyphae *c-e* arising from thallus *a*, or between the four hyphae *f-i* arising from thallus *b*, but the hypha *e* from thallus *a* was found paired with the hypha *g* from thallus *b*. Likewise in figure 6, *H*, showing portions of two thalli, *a* and *b*, no pairing could be made out between any of the five reproductive hyphae *c-g* arising from thallus *a*, but one of them, *f*, had paired with a hypha, *h*, coming from thallus *b*. Again in the reproductive apparatus shown in figure 6, *I*, the two empty thalli *a* and *b* are seen to have contributed the paired hyphae *c* and *d*, respectively, although the three additional hyphae *e-g* arising from thallus *b* have not paired with one another. Further, in the unit of sexual

apparatus shown in figure 6, *J*, the empty thalli *a* and *b* have contributed the paired zygophoric hyphae *c* and *d*, respectively; while similarly in the unit of sexual apparatus illustrated in figure 6, *K*, the empty thallus *a* and the empty infective body *b*, separate from it, have given off the paired zygophoric hyphae *c* and *d*, respectively.

The paired zygophoric hyphae, on making apical contact with each other and also with the host animal's pellicle (FIG. 6, *G-K*: *p*), secrete at their tips some yellow adhesive material. Thus anchored securely to the pellicle, they break jointly through this envelope, and then elongate externally side by side for distances varying commonly from 5 to 35 μ before fusing at the tip. As a rule, and more especially where elongation takes place under the surface of a culture, the hyphae follow rather straightforward courses (FIG. 6, *G*). Where external elongation takes place on the surface of a culture the zygophoric hyphae in some instances wind spirally about one another, their direction of rotation then being indiscriminately either right-handed (FIG. 6, *H*) or left-handed, rather than consistently left-handed like the rotation in the thallodic coils.

When their elongation ceases the paired hyphae fuse apically and begin to bud forth a globose body (FIG. 6, *G*, *j*; *H*, *i*) directly at the place of union, or terminally on a short stout prolongation usually not more than 2 μ in length. Meanwhile a cross-wall (FIG. 6, *G*, *s*; *H*, *s*; *J*, *s*; *K*, *s*) proximally delimiting a gametangium makes its appearance in each of the paired hyphae and usually in a position well within the host pellicle. The globose body increases gradually in size as it receives protoplasmic material from the gametangia. When the gametangia have given all their contents, it usually has reached a definitive diameter of approximately 15 μ (FIG. 6, *I*, *h*; *J*, *c*; *K*, *c*) and then apparently becomes delimited at or near the base by a retaining wall. The protoplast of the subspherical zygosporangium thus set off soon shrinks away from the enveloping wall, which thereupon usually collapses slightly to present a somewhat wavy contour (FIG. 6, *L*). Later the protoplast takes on an angular shape (FIG. 6, *M*), preparatory evidently to the formation of a yellowish zygospore wall with a pronouncedly warty or stellate outline. Within this wall the originally granular contents round up into a spherical living cell (FIG. 3, *C-P*; FIG. 6, *N*). At maturity this cell seems to be constituted, as a rule, of an outer granu-

lar layer surrounding a more nearly homogeneous reserve globule and having imbedded in it a somewhat flattened refringent body. The internal organization of the resting zygospore here as in the several congeneric forms and in most other members of the Zoöpagaceae would seem to correspond to the unitary organization familiar in the resting oospores of many oomycetes, including most species of *Pythium* and all species of *Aphanomyces*.

As the individual zygospores are produced close to the host pellicle in numbers ranging usually from 10 to 25 (FIG. 5, *K*, *a-y*)—the greatest number noted was 37—and are of durable character, they arrest attention by their clustered arrangement for months after all vestiges of their protozoan hosts have vanished from sight. In some plate cultures many were observed being invaded and destroyed by a hyphomycete that developed mainly through parasitism on the oospores of *Pythium mamillatum*. Though a mycelial connection could not be traced the hyphomycete in question was most probably *Trinacrium subtile* Fres., since the distinctive conidia of that oospore parasite were present in some abundance. The same hyphomycete also parasitized many specimens of the testaceous rhizopod *Arcella vulgaris* Ehrenb. that were distributed on the surface of the agar, and further invaded numerous globose cysts which had been produced in the cultures by a member of the Myxobacteriaceae.

A term having reference to its predominantly binary vegetative development is deemed suitable as a specific name for the new fungus.

Endocochlus binarius sp. nov.

Corpus intromissum per tubulum germinationis nuciforme, plerumque 12–20 μ longum, 6–10 μ crassum, basi acutulum, apice primo rotundum, mox ex apice vulgo 2 (interdum 1 et rarius 3) hyphas assumentes pullulans, postea aliquando 1 vel 2 hyphas genitabiles (hyphas conidiiferas vel zygosporiferas) uspiam emittens; hyphis assumentibus incoloratis, prope basim 2–4.5 μ crassis, sursum leniter quandoque usque 8 μ latescentibus, usque 200 μ longis, in spiram semel vel bis vel ultra quam ter ad modum caulis Humuli lupuli cochleatim convolutis, nunc simplicibus nunc semel usque ter repetite bifurcis, animal debilitato vel moribundo prope originem ex latere convexo 1–8 hyphas genitabiles emittentibus. Hyphae conidiiferae per pelliculam animalis solitatim erumpentes, extra animal procumbentes et paene rectae, simplices vel parve ramosae, incoloratae, saepe 2.5–3 mm. longae, plerumque 2.5–3.3 μ crassae, primo continuae et protoplasmatis repletae, post auctum conidiorum

inanes et septatae; conidiis inter se saepius 75–125 μ distantibus, incoloratis, erectis, post disjunctionem deorsum subter septo inferiore membrana circulari inani 1–3 μ longa praeditis, vulgo in cellula viventi et appendice vacua terminali consistentibus; cellula viventi ellipsoidea vel fusiformi, 20–45 μ longa, 5.7–9 μ crassa; appendice vacua 2–10 μ longa, deorsum plerumque 1–1.5 μ crassa, apice 0.6–0.8 μ crassa. Hyphae zygosporiferae 2–4.5 μ crassae, ambae ex aliis hyphis assumentibus oriundae, apice ad pelliculam animalis communiter inhaerentes, mox ex pellicula communiter erumpentes, communiter 5–35 μ in longitudine interdum spiraliter interdum recto crescentes, denique gametangiis terminalibus saepius 20–50 μ longis seclusis in apice conjungentes et zygosporangium ex junctione gignentes; zygosporangio globoso, plerumque 12–17 μ in diametro, membrana ejus in maturitate circum zygosporam laxe collapsa; zygospora flavida globosa, 11–15 μ crassa, membrana in maturitate valde verrucosa, cellulam viventem sphaeralem 8–10.5 μ crassam circumdante.

Amoebam papyraceam enecans habitat in foliis Quercus putrescentibus prope Greensboro, North Carolina.

Infective body formed on intruded germ tube usually elongated ellipsoidal, minutely protruding at the proximal end, rounded at the distal end, usually 12–20 μ long and 6–10 μ wide, from opposite positions on the distal end soon putting forth 2 (sometimes 1 and more rarely 3) assimilative hyphae, and later, on disablement or death of animal host, sometimes extending from indeterminate positions 1–2 reproductive (*i.e.*, conidiiferous or zygophoric) hyphae. Assimilative hyphae colorless, up to 200 μ long, near their attachment usually 2–4.5 μ wide, usually widening rather gradually to attain more distally sometimes a diameter of 8 μ, convolved in a helicoid spiral of 1–3.5 turns with left-handed rotation, simple or once-dichotomous or successively bifurcated 2–3 times; after disablement or death of host animal giving rise proximally from the convex side to reproductive hyphae in numbers ranging from 1 to 8. Conidiiferous hyphae erupting individually from the animal's pellicle to elongate procumbently in rather straightforward courses for distances frequently of 2.5–3 mm. at a width between 2.5 and 3.3 μ, simple or sparingly branched, at first continuous and filled with protoplasm, but becoming empty and septate in producing erect conidia at intervals frequently of 75–125 μ. Conidia colorless, consisting individually of an elongated ellipsoidal or spindle-shaped living cell, 20–45 μ long and 5.7–9 μ wide, surmounted usually by an empty tapering appendage 2–10 μ long, 1–1.5 μ wide proximally, and tapering to a width of 0.6–0.8 μ near the tip; the individual spore usually becoming detached a short distance below its basal septum, and thus bearing proximally an empty collar-like membranous prolongation 1–3 μ long. Zygophoric hyphae 2–4.5 μ wide, each pair arising from separate assimilative structures, the

two usually making close contact at their apices before breaking jointly through the host pellicle to elongate together outside 5–35 μ usually in a straightforward course but sometimes winding spirally about one another, then (terminal gametangia often 20–50 μ long having been delimited) fusing at the tip and budding forth a zygosporangium from the place of union. Zygosporangium globose, usually 12–17 μ in diameter, its wall at maturity collapsing loosely about the zygospore. Zygospore subspherical, 11–15 μ in diameter, slightly yellowish, its wall at maturity presenting a somewhat stellate profile around a spherical protoplast 8–10.5 μ in diameter.

Destroying *Amoeba papyracea* it occurs in decaying Quercus leaves near Greensboro, North Carolina.

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EXPLANATION OF FIGURES

FIG. 1. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba papyracea* in active condition though under multiple attack by the fungus, with

the fungus in various stages of development: *a*, adhering conidium with a funnel-shaped germinative outgrowth that has penetrated the animal's pellicle and is forming terminally an infective body in the host protoplasm; *b*, adhering conidial envelope that has been emptied by movement of its contents through the tapering germ tube into the terminal infective body, which now is fully formed and delimited at its base by a retaining wall; *c*, newly detached infective body; *d*, *e*, two infective bodies, each of which has begun to grow out distally into a single thallodic coil; *f*, an infective body that has grown out distally into a single thallodic coil of two turns; *g*, infective body that is budding out in opposite positions at its distal end, to begin the development of two thallodic coils; *h*, similar infective body whose two distal outgrowths are somewhat longer and thus show in the orientation of their expanded tips the left-handed rotation characteristic of the vegetative stage of the fungus; *i*, infective body with two regularly convolved thallodic coils, each coil consisting of one and one-half turns; *j*, infective body with two less regularly convolved thallodic coils, each consisting of approximately one and one-fourth turns; *k*, empty infective body that has produced two regularly convolved thallodic coils, each of approximately two turns and each delimited proximally by a retaining wall; *l*, infective body with two regularly convolved thallodic coils shown endwise, each coil consisting of about one and one-half turns; *m*, infective body with two regularly convolved thallodic coils, each of which is bifurcated about one and one-half turns from its origin; *n*, nucleus of animal host; *o*, large infective body that has put forth three thallodic outgrowths, each disposed in a left-handed coil of about one-half turn; *v*, contractile vacuole of animal host; *w*, encysted specimen of *Euglypha laevis* ingested by animal host. *B–U*, Conidia of usual make-up, showing ordinary variations in size and shape, and in length of empty beak. *V*, *W*, Conidia with short apical beaks that apparently are not empty. *X*, Conidium with small empty apical beak. *Y*, Conidium including at the base a portion of the parent hypha. *Z*, Conidium lacking an apical beak, and at the base including a portion of the parent hypha.

FIG. 2. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba papyracea* still actively motile though under multiple attack by the fungus: *a*, infective body that has grown out distally to form a young thallodic coil of one-half turn; *b*, infective body with two regularly convolved thallodic coils, each consisting of approximately a three-fourth turn; *c*, infective body with two somewhat irregularly convolved thallodic coils, each comprising approximately one turn; *d*, infective body (viewed from the proximal end) with two thallodic coils, each composed of one turn; *e*, infective body with two regularly convolved thallodic coils, each comprising slightly more than one turn; *f*, infective body (viewed from the distal end) bearing two regularly convolved thallodic coils, each of slightly more than one turn; *g*, infective body (likewise viewed from distal end) bearing two regularly convolved thallodic coils, each comprising about one and one-half turns; *h*, empty collapsed membranous envelope of an infective body, to which are attached two regularly convolved thallodic coils, each delimited proximally by a retaining wall and comprising two turns; *i*, infective body that has grown out into a single thallodic coil, which bifurcated one and three-fourth turns from its

origin; *j*, *k*, infective bodies (in oblique view), each with two thallodic coils, one of them being simple, the other one being bifurcated about one-half turn from its origin; *n*, nucleus of animal host; *v*, contractile vacuole of host; *w*, ingested specimen of *Euglypha levis*. *B–M*, Conidia with various lateral modifications ranging from 1 to 5 in number, each resulting from frustrated infective germination; in *C* the broad adhesive appressorium has attached to it a portion of pellicle and protoplasm torn from an animal in its successful escape; in *D* the distally evacuated germ tube is shown encased in a remnant of protoplasm from an animal that escaped infection after deep penetration by the fungus.

FIG. 3. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba papyracea* still in motile condition though under multiple attack by the fungus: *a*, infective body that has grown out distally into a single thallodic prolongation, which began coiling later than is usual; *b*, *c*, infective bodies that have each grown out into a single thallodic coil of a three-fourth turn; *d*, *e*, infective bodies, each of which has grown out into an irregularly convolved thallodic coil of one and one-half turns; *f*, *g*, infective bodies, each of which has grown out into a single thallodic coil that is bifurcating after describing one and three-fourth turns; *h*, infective body with two thallodic coils, each comprising a single turn; *i*, infective body with two thallodic coils of a single turn, the coil attached on the left showing departure from the usual in having taken a course on the same side of the infective body as the coil attached on the right; *j*, single coiled bifurcate thallus comprising two and one-half successive turns, which is not attached to any visible infective body; *k*, infective body bearing two thallodic coils, only one of which is shown with its two and one-half successive turns and its two bifurcations; *n*, nucleus of animal host; *v*, contractile vacuole of animal. *B*, Infective body with two thallodic coils, each of them once bifurcate and each consisting of two and one-half successive turns: *a*, the complete assimilative structure, with all parts in place; *b*, the infective body with the one thallodic coil underneath it; *c*, the infective body with a proximal portion of each thallodic coil; *d*, the infective body with the one thallodic coil overlying it. *C*, *D*, Portions of empty paired zygophoric hyphae whereon are borne globose zygosporangia, each containing a zygospore somewhat immature in the organization of its protoplast. *E–P*, Zygosporangia, each containing a zygospore having a prominently warty envelope and a subspherical protoplast of mature structure.

FIG. 4. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Specimen of *Amoeba papyracea* still in active condition though under multiple attack by the fungus: *a*, empty membranous envelope of infective body bearing two regularly convolved thalli, each comprising two and one-half turns and each delimited proximally by a retaining wall; *b*, infective body bearing two regularly convolved unbranched thallodic coils, each consisting of one and three-fourth turns; *c*, thallodic coil, comprising two and one-half successive turns and showing two bifurcations, which no longer is attached to any visible infective body; *d*, well developed thallodic coil, convolved in three to three and one-half successive turns and showing three successive bifurcations, which lies over its companion whereof only the eight terminations directly exposed

to view are shown, their contours being drawn with stippled lines; *n*, nucleus of animal host; *v*, contractile vacuole of animal. *B*, Portion of a continuous prostrate conidiiferous hypha showing a conidium, *a*, in early stage of development, and a cluster of vacuoles, *b*, resulting from initial loss of hyphal contents. *C*, Portion of prostrate conidiiferous hypha showing a young conidium, and several partitions formed on progressive evacuation of hyphal parts. *D*, Portion of prostrate conidiiferous hypha showing a conidium in more advanced stage of development. *E*, Portion of empty prostrate hypha with a fully developed conidium. *F–U*, Fully developed conidia showing usual variations in size and shape of both the living cell and the empty apical beak. *V*, Conidium in which the apical beak is reduced to a thickened cap.

FIG. 5. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 500 throughout. *A*, *B*, Two specimens of *Amoeba papyracea* no longer capable of locomotion owing to advanced infection of each by approximately ten thalli variously intertangled and massed in a confused clew; the two animals, despite marked degeneration of their nuclei, *n*, and reduction of their protoplasm to about one-tenth of the normal quantity, still showing life in continued operation of their contractile vacuoles, *v*. *C*, Portion of continuous conidiiferous hypha showing three conidia, *a–c*, in early stage of development; owing to lack of space the hyphal portion is shown in two parts connecting at the point *z*. *D*, Portion of conidiiferous hypha with empty intervals that were evacuated and partitioned owing to progressive movement of contents into the three growing conidia *a–c*; the hyphal portion being shown, from lack of space, in two parts connecting at the point *z*. *E*, Short portion of conidiiferous hypha on which two conidia, *a* and *b*, are being formed. *F*, *G*, Largely evacuated portions of conidiiferous hypha, each showing a conidium, *a*, in advanced stage of development, and a fully developed conidium, *b*. *H–J*, Random assortment of conidia, *a–z*, showing variations in size and shape of the living cell and of the apical beak, as well as in the length of the basal membranous prolongation; a few examples (*H*, *t*; *I*, *a*, *b*, *d*, *e*) showing variable extension of protoplasm into the parent hypha. *K*, Wrinkled collapsed pellicle of a large specimen of *Amoeba papyracea* surrounded by twenty-five zygosporangia of the parasite, *a–y*, each containing a prominently warty zygospore with a spherical protoplast in mature resting condition.

FIG. 6. *Endocochlus binarius*, drawn to a uniform magnification with the aid of a camera lucida; × 1000 throughout. *A*, Infective body with a twice bifurcated thallodic coil of two successive turns; one reproductive hypha, *a*, is growing out directly from the infective body, and another, *b*, from the proximal portion of the coil. *B*, Infective body with two thallodic coils, each comprising one and three-fourth turns and bifurcating once; two reproductive hyphae, *a* and *b*, are growing out from the infective body, while two others, *c* and *d*, are being extended from the basal portions of the coils. *C*, Proximal portion of a thallodic coil from which eight reproductive hyphae, *a–h*, have been extended; the infective body from which the coil originated is no longer visible. *D*, Empty membranous envelope of an infective body and of proximal portions of two thallodic coils; three empty reproductive hyphae, *a–c*, are shown arising from one coil, and four similar hyphae, *d–g*,

are shown arising from the other. *E*, Branched helicoid thallus with six terminal elements, each of which is partly or wholly emptied and provided with a retaining wall; below these walls the contents show pronounced vacuolization; four reproductive hyphae, *a–d*, are given off from the proximal turn. *F*, Tips of two zygophoric hyphae, *a* and *b*, making contact on the inner side of the host pellicle, *p*. *G*, Two helicoid thalli, *a* and *b*, the former having put forth three reproductive hyphae, *c–e*, the latter having put forth four such hyphae, *f–i*; after the hyphae *e* and *g* had paired by tip-to-tip contact on the inner surface of the host pellicle, *p*, they grew through the pellicle side by side for a distance of 20 μ and then fused apically to begin development of the partly grown zygosporangium, *j*; a septum, *s*, had in the meantime been formed in each hypha to delimit the paired gametangia; the hypha *d* is present as a frustrated supernumerary zygophore. *H*, Two helicoid thalli, *a* and *b*, that have put forth the reproductive hyphae *c–h*; the hypha *f* (from thallus *a*) having paired with hypha *h* (from thallus *b*) by apical contact on the inner surface of the host pellicle, *p*, the two broke jointly through the pellicle and elongated outside for a distance of 25 μ while winding about one another before they fused apically to give rise to the globose zygosporangium *i*; a septum, *s*, having meanwhile been formed in each zygophoric hypha to delimit the paired gametangia. *I*, Two empty helicoid thalli, *a* and *b*, from which were put forth the reproductive hyphae *c–g*; after the hyphae *c* and *d* (originating from thalli *a* and *b*, respectively) had paired by making contact on the inner surface of the host pellicle, *p*, the two erupted jointly to elongate externally for a distance of 5 μ, and then fused apically to form the zygosporangium *h*, now fully grown and delimited at the base. *J*, Parts of two empty thalli, *a* and *b*, from which were extended the zygophoric hyphae *c* and *d*, respectively; the two hyphae, after becoming paired by adhering together apically on the inner side of the host pellicle, *p*, having erupted and grown externally side by side for a distance of 25 μ, then having fused apically to form the zygosporangium *c*, now fully grown and delimited at the base; *s*, septa delimiting the paired gametangia. *K*, Portion of empty thallus, *a*, and an empty infective body, *b*, from which were extended the zygophoric hyphae *c* and *d*, respectively; these hyphae, after becoming paired through mutual adhesion on the inner side of the host pellicle, *p*, having erupted together and grown externally side by side for a distance of 15 μ, then having fused apically to form the zygosporangium, now fully grown and delimited at the base; *s*, septum delimiting the gametangium supplied by hypha *c*. *L*, *M*, Two zygosporangia whose protoplasts have shrunken away from the slightly collapsed envelopes to form young zygospores. *N*, Slightly collapsed zygosporangial envelope surrounding a mature zygospore with boldly verrucose wall and subspherical protoplast.

A SECOND CONTRIBUTION TO THE KNOWLEDGE OF THE USTILAGINALES OF CHINA [1]

LEE LING

(WITH 2 FIGURES)

The present contribution deals primarily with the Ustilaginales collected in Taiwan (Formosa) of South China. The materials studied were obtained from the herbarium of the Taiwan Agricultural Research Institute, the herbarium of the National University of Taiwan, and the personal collection of Y. Hashioka.

The first record of Ustilaginales from Taiwan was made by P. Hennings (1). He recorded three species, among which *Ustilago digitariae* (Kze.) Rabh. has not been reported since under that name although it is probable that it is the same species now known as *Sorosporium formosanum* Saw. Later both Sawada and Ito studied this group of fungi from this region. All the species recorded by them have been examined by the writer and several of the species described by them have been reduced to synonymy, with the result that the total number of species previously reported from the island has been reduced to forty-six.

Since several species described by Sawada after 1935 were not provided with Latin diagnoses, these are supplied here to legitimatize their publication. These diagnoses, however, are chiefly based upon the observations of the writer rather than upon translations from the original.

TILLETIACEAE

1. **Tilletia alopecuri** (Saw.) Ling, comb. nov. (FIG. 1, a; and 2, a)
Entyloma alopecuri Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. 2: 86. 1922.

Sori in the ovaries, infecting most of the spikelets, inconspicuous, hidden completely by the enveloping glumes and each covered

[1] The first of this series was published in Mycological Papers, Kew Mycological Institute, No. 11. 1945.

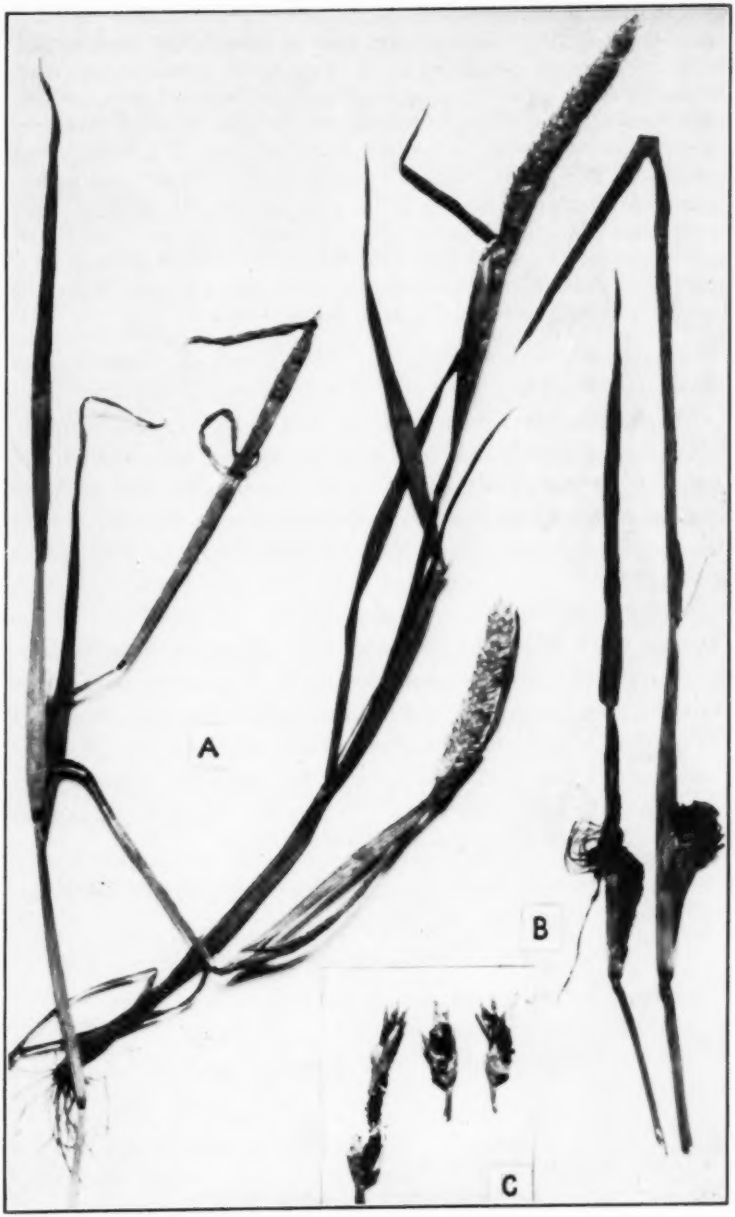

FIG. 1. Ustilaginales of China.

by a thin membrane of host tissue, broadly elliptical with a tapering apex, approximately 1 mm. long; also in leaves, leaf sheaths, and internodes of culms, forming short or elongate, yellow striae, later becoming dark brown, the infected parts of culms hypertrophied, often causing curvature and bulging of the culms; chlamydospores light yellow, spherical to subspherical, enclosed in a hyaline envelope, 17–25.5 μ diam., with a mean of 22.2 μ, covered with prominent spiny scales measuring 2–3 μ high by 2.5–3.5 μ wide at base, germination by a short promycelium with an apical whorl of 16–18 primary sporidia; sterile cells almost hyaline, 12–16.5 μ, walls 1.5 μ thick; conidia formed on surface of infected vegetative tissues of the host, hyaline, smooth, 14–35 × 5–8 μ.

On *Alopecurus geniculatus* L., Taipeh, coll. K. Sawada (cotype), Jan. 19, 1914.

This fungus infects the ovaries as well as the vegetative organs of the host. Sawada, in his original description, apparently overlooked the presence of infection in the ovaries and was probably also impressed by the occurrence of conidia in nature, which is very unusual for *Tilletia*. Those facts led him to place it in the genus *Entyloma*.

In Arkansas, U. S., a similar fungus, described as *Tilletia youngii* Clint. & Zundel, was found on *Alopecurus carolinianus*. The type of that species agrees well with *T. alopecuri* in both the symptoms caused and the general appearance of spores, though in its original description the presence of sori on leaves and leaf sheaths was neglected. *T. youngii*, however, has larger spores measuring 19.5–27 μ diam. with an average of 25.1 μ and coarser spiny scales which are 2.5–4.7 μ wide at base.

2. NEOVOSSIA HORRIDA (Takah.) Padw. & Azmatullah Khan, Mycol. Papers **10**: 2. 1944.

On *Oryza sativa* L., Taipeh, coll. K. Sawada, Oct. 28, 1926.

3. ENTYLOMA AUSTRALE Speg., Anal. Soc. Ci. Argent. **10**: 5. 1880.

On *Physalis angulata* L., Yüli, Hualienhsien, coll. K. Sawada, May 13, 1909.

4. ENTYLOMA BIDENTIS P. Henn., Pflanzenw. Ost-Afrikas C. **5**: 49. 1895.

On *Bidens pilosa* L., Taipeh, coll. K. Sawada, June 5, 1942.

5. **Entyloma eleocharidis** (Saw.) Ling, comb. nov. (FIG. 2, c)
Ustilago eleocharidis Saw., Taiwan Agr. Res. Inst. Rept. **85**: 39. 1943.

Soris in culmorum partibus superioribus, circularibus vel irregularibus, plumbeo-nigris, 0.1–1 mm. longis; sporis in columnis paralellibus catenatis inter epidermides evolutis, globosis usque subglobosis, saepe angularibus, 9–12 × 7–10.5 μ, episporio tenui, dilute olivaceo-brunneo, levi.
In culmis *Eleocharidis dulcis* Trin.

Sori on the upper parts of the culms, circular or irregular, lead black, 0.1–1 mm. long; spores embedded permanently in the host tissues between the upper and lower epidermis, arranged in parallel columns, globose to subglobose, often angular, 9–12 × 7–10.5 μ, epispore thin, smooth, light olivaceous brown.
On *Eleocharis dulcis* Trin., Taipeh, coll. K. Sawada (*type*), Nov. 16, 1934.

Although the taxonomic position of this species cannot be ascertained without knowledge of its spore germination, the appearance of sori and spores indicates that it is more likely to be an *Entyloma* than a *Ustilago*. A similar fungus, *Entyloma parvum* Davis, occurs in North America on the same host genus. Its sori appear on the culms as small fusiform swellings, while *E. eleocharidis* causes no perceptible hypertrophy. Besides, *E. parvum* has spores smaller in average, but more variable in size and shape, than *E. eleocharidis*. The spores of the former also have a thicker and darker epispore.

6. ENTYLOMA MICROSPORIUM (Ung.) Schroet., in Rabh. Fungi Eur. n. 1872. 1874.

On *Ranunculus vernyi* Franch. & Sav., Taipeh, coll. K. Sawada, May 13, 1917; also Apr. 4, 1927.

7. ENTYLOMA SENECIONIS Saw., Jour. Taihoku Soc. Agr. and For. **7**: 27. 1934. (Fig. 2, b)

Sori in the leaves, forming chiefly angular brownish spots, limited by veins, 1–2 mm. long; spores globose to ellipsoid, light yellow, 10–13 μ diam., or 14–17 × 8–13 μ, epispore smooth, 0.6–1 μ thick.
On *Senecio formosana* Kitam., Mt. Nêngkao, Taichung, coll. K. Sawada (*type*), Aug. 4, 1928.

This species is probably identical with *Entyloma bavaricum* Sydow.

8. ENTYLOMA ORYZAE H. & P. Syd., Ann. Myc. **12**: 197. 1914.
Ectostroma oryzae Saw., Taiwan Agr. Rev. no. 63, p. 107. 1912.

On *Oryza sativa* L., Taipeh, coll. K. Sawada, Oct. 20, 1926.

9. BURRILLIA AJREKARI Thirumal., Mycologia **39**: 607. 1947.
Stereosorus monochoriae Saw., Taiwan Agr. Res. Inst. Rept. **85**: 45. 1943. (*nomen nudum*)

On *Monochoria vaginalis* Presl, Taipeh, coll. K. Sawada (type of *S. monochoriae*), July 8, 1942.

The genus *Stereosorus* was founded on the assumption that the sorus consists entirely of fertile spores, but the specimen shows clearly the presence of sterile parenchymatous cells intermixed with the spores.

10. DOASSANSIA OPACA Setch., Proc. Amer. Acad. **26**: 15. 1891.

On *Sagittaria trifolia* L., Taipeh, coll. H. Sueda, May 28, 1919.

USTILAGINACEAE

11. USTILAGO AVENAE (Pers.) Rostr., Overs. K. Danske Vid. Selsk. Forh. **1890**: 13. 1890.

On *Avena sativa* L., Taipeh, coll. K. Sawada, June 4, 1909.

12. USTILAGO CRAMERI Koern., in Fckl. Jahrb. Nass. Ver. Nat. **27–28**: 11. 1873.

On *Setaria italica* Beauv., Taitung, coll. K. Sawada, May 29, 1911.

13. USTILAGO CRUS-GALLI Tracy & Earle, Bull. Torrey Bot. Club **22**: 175. 1895.

On *Echinochloa crusgalli* Beauv., Taipeh, coll. K. Sawada, June 17, 1927.

14. USTILAGO CYNODONTIS P. Henn., Bull. Herb. Boiss. **1**: 114. 1893.

On *Cynodon dactylon* Pers., Taipeh, coll. K. Sawada, Jan. 17, 1944.

Zundel (6) states that the name of this species should be written *U. cynodontis* (Pass.) Curzi. Under the provisions of article 58 of the International Rules of Botanical Nomenclature, however, the name of P. Hennings is the legitimate one.

15. USTILAGO EMODENSIS Berk., in Hooker's Jour. Bot. **3**: 202. 1851.

Ustilago treubii Solms Laubach, Ann. Jard. Bot. Buitenz. **6**: 79. 1886.

Elateromyces treubii Bub., České Houby **2**: 33. 1912.

Ustilago rosulata Syd., Ann. Myc. **10**: 77. 1912.

Farysia emodensis (Berk.) Syd., Ann. Myc. **17**: 42. 1919.

Liroa emodensis (Berk.) Cif., Nuo. Giorn. Bot. Ital. N. S. **40**: 263. 1933.

On *Polygonum chinense* L., Taipeh, coll. K. Sawada, Mar. 23, 1919; Tsaosan, Taipeh, coll. Y. Hashioka, Feb. 1932.

Bubák transferred this species to *Elateromyces* because of the presence in the sorus of remnants of host tissue which resemble "elaters" superficially. Sydow accordingly placed it in *Farysia*. Ciferri established the genus *Liroa* based on the same fungus. His genus is separated from *Farysia* by its habit of forming galls on various parts of *Polygonum* and its spore formation in lysigenous cavities. These features, however, do not form a sound basis for generic distinctions and especially fail to separate the genus *Liroa* from *Ustilago*. Ciferri also believed that the sporogenesis in this fungus was typical of the Graphiolaceae. On the contrary, Mundkur and Thirumalachar (**4**) ascertained that the spore formation conformed to that of the Ustilaginaceae. Their studies on the anatomy of the tumor led them to emphasize the formation of an inverted lunate bed of sporogenous tissue restricted to the apical region of the tumor as the most characteristic feature of the genus. This feature, however, is by no means constant even in the species concerned. The fungus attacks the inflorescence as well as the stem, but such inverted lunate beds of sporogenous tissue can be traced out only in the conical outgrowths on the stem. If the fungus manifests itself only in the floral parts, as is often the case, this diagnostic feature will not be present. Moreover, the occurrence of "elater"-like strands of host tissue is not unusual in other species of the Ustilaginaceae. Certain collections of ovaricolous *Ustilago* on *Polygonum* do have such host remnants intermixed with spores. In *Sorosporium tanganikeanum* Zundel, the "yellowish shreds" mentioned in its original description (**7**) are also very

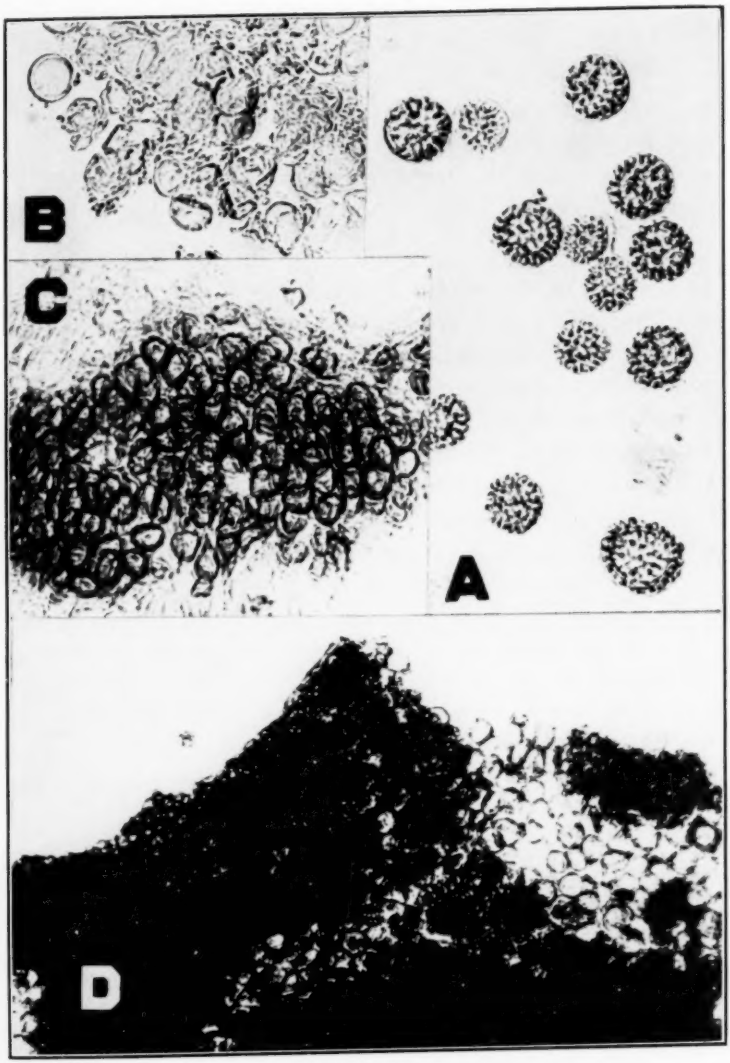

FIG. 2. Ustilaginales of China.

similar to those found in *U. emodensis*. Taking the foregoing into consideration, the proper taxonomic position for the fungus in question is in *Ustilago*, in which genus it was originally placed. Consequently the generic name *Liroa* should be relegated to the synonymy of *Ustilago*.

The spores of this species have been described as smooth, verruculose, or reticulate. All the specimens examined possess spores with very fine and regular reticulations, measuring approximately 0.7–1 μ in width and appearing as verruculae under lower magnifications. In addition to Taiwan collections, the following were included in the present study: PHILIPPINE ISLANDS: Mt. Apo, S. E. Mindanao, coll. R. S. Williams, Apr. 1, 1905; Mt. Puloz, Benguet, Luzon, coll. M. S. Clemens 5127, Feb. 24–27, 1925; Baguio, Benguet, Luzon, coll. M. S. Clemens 51798, Mar. 28, 1935. CEYLON: Hakgala, coll. T. Petch, Aug. 1912 (Syd., Fungi Exot. Exs. 73).

16. USTILAGO ESCULENTA P. Henn., Hedwigia **34**: 10. 1895.

On *Zizania latifolia* Turcz., Taipeh, coll. Y. Hashioka, Nov. 18, 1933; coll. K. Sawada, Nov. 2, 1927.

17. USTILAGO HORDEI (Pers.) Lagerh., Mitt. d. Badischen Bot. Ver. p. 70. 1889.

On *Hordeum sativum* Jessen, Taipeh, coll. K. Sawada, Mar. 8, 1911.

18. USTILAGO KOLLERI Wille, Bot. Notiser **1893**: 10. 1893.

On *Avena sativa* L., Taipeh, coll. K. Sawada, June 4, 1909.

19. USTILAGO KUSANOI Syd., Mem. Herb. Boiss. **4**: 4. 1900.

On *Miscanthus sinensis* Anders., Taipeh, col. K. Sawada, Mar. 25, 1926.

20. USTILAGO NEGLECTA Niessl. in Rabh. Fungi Eur. n. 1200. 1868.

On *Setaria glauca* Beauv., Taichung, coll. Y. Fujikuro, Aug. 8, 1911.

21. USTILAGO NUDA (Jens.) Rostr., Tidsskrift f. Landökonomi **8**: 745. 1889.

On *Hordeum sativa* Jessen, Taipeh, coll. K. Sawada, Mar. 10, 1910.

22. USTILAGO SCITAMINEA Syd., Ann. Myc. **22**: 280. 1924.

On *Saccharum officinarum* L., Hsinchu, coll. K. Sawada, June 23, 1929.

23. USTILAGO SHIMADAE Saw., Taiwan Agr. Res. Inst. Rept. **87**: 34. 1943.

Soris axem floralem omnino destruentibus et eum in structuram longam, curvatam flagelliformem, membrana albida delicata e contextu hospitis composita tectam, circa 25 cm. longam transformantibus; sporis plerumque globosis usque subglobosis, 4.4–6.5 μ in diam., episporis tenuibus, glabris, pallide olivaceo-brunneis.

In Gramineis indeterminatis.

Sori entirely destroying the floral axis, transforming it into a long, curved flagelliform structure, covered by a whitish delicate membrane of host tissue, approximately 25 cm. long; spores chiefly globose to subglobose, 4.4–6.5 μ diam., epispore thin, smooth, light olivaceous brown.

On an undetermined Grass, Taipeh, coll. Y. Shimada (*type*), Apr. 3, 1911.

The host was noted as *Andropogon?* or *Arundinella?* on the label of the type. The fungus differs from *Ustilago arundinellae-hirtae* Ito, described from Japan, only slightly in spore size.

24. USTILAGO SHIRAIANA P. Henn., in Engl. Bot. Jahrb. **28**: 260. 1901.

On *Phyllostachys makinoi* Hayata, Tanshui, Taipeh, coll. K. Sawada, Apr. 16, 1927.

25. USTILAGO SPARSA Underw., Bull. Torrey Bot. Club **24**: 86. 1897.

On *Dactyloctenium aegypticum* Willd., Anping, Tainan, coll. K. Sawada, Nov. 4, 1908.

26. USTILAGO SPHAEROGENA Burr., in Ell. & Ev. N. Am. Fungi n. 1892. 1887.

On *Echinochloa crus-galli* Beauv., Taipeh, coll. K. Sawada, July 5, 1924.

27. USTILAGO TRITICI (Pers.) Rostr., Overs. Kong. Danske Vidensk. Forh. **1890**: 15. 1890.

On *Triticum aestivum* L., Taipeh, coll. Y. Fujikuro, Apr. 2, 1917.

28. USTILAGO TUBERCULIFORMIS Syd., Ann. Myc. **1**: 22. 1903.
Ustilago foliorum Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 88. 1935.

On *Polygonum runcinatum* Hamilt. ex D. Don. (= *P. morrisonense* Hayata), Mt. Nêngkao, Taichung, coll. K. Sawada, Aug. 4, 1928.

This species was first collected by A. Henry in Hupeh, Central China. Efforts to locate the type have been unsuccessful. The only other record (**5**) is from Darjeeling, India on *Polygonum chinense*. In this Indian collection made by W. McRae in 1909, the sori occur in the midrib of the leaf, forming conspicuous tumors 3–7 mm. in length. The spores are light flesh color, 12–14.6 × 11–14.5 μ diam., with violet reticulations of 1.5–2 μ deep by 2–4 μ wide. The Taiwan collection available for the present study was from the same locality as the type of *U. foliorum*. The sori are in the leaves causing slight thickening only. It agrees with the original description of *U. tuberculiformis* even better than the Indian specimen.

29. USTILAGO UTRICULOSA (Nees) Tul., Ann. Sci. Nat. Bot. III. **7**: 102. 1847.

On *Polygonum barbatum* L., Chia-i, coll. K. Sawada, Nov. 9, 1909; on *Polygonum caespitosum* Bl. var. *longisetum* (De Bruyn) Steward, Taipeh, coll. K. Sawada, May 4, 1920; also Dec. 3, 1923; on *Polygonum lapathifolium* L., Taipeh, coll. Y. Hashioka, Apr. 15, 1932; on *Polygonum macranthum* Meisn. (= *P. japonicum* Meisn. var. *conspicuum* Nakai), Shinsha, coll. F. Onuma, Oct. 4, 1930.

Liro (**2**) has seen a collection on *Polygonum barbatum* from Taiwan and referred it to *Ustilago cordai* Liro.

30. SPHACELOTHECA CRUENTA (Kuehn) A. A. Potter, Phytopath. **2**: 98. 1912.

On *Sorghum vulgare* Pers., Taipeh, coll. K. Sawada, June 20, 1911.

31. SPHACELOTHECA HYDROPIPERIS (Schum.) D. By., Verg. Morph. u. Biol. d. Pilze, p. 187. 1884.

Ustilago polygoni-senticosi P. Henn., *in litt.*

Sphacelotheca polygoni-senticosi (P. Henn.) Miy. & Tak., Trans. Sapporo Nat. Hist. Soc. **14**: 90. 1935.

On *Polygonum caespitosum* Bl., Taipeh, coll. K. Sawada, May 24, 1917; Taroko, Hualienhsien, coll. T. Hosokawa, Aug. 13, 1932; on *Polygonum caespitosum* Bl. var. *longisetum* (De Bruyn) Steward, Taipeh, coll. Y. Fujikuro, Apr. 3, 1915; Taichung, coll. Y. Hashioka, July 1, 1936; on *Polygonum senticosum* (Meisn.) Franch. & Sav., Wanyülin, Hualienhsien, coll. K. Sawada, May 18, 1919.

32. **Sphacelotheca isachnes** (Syd.) Ling, comb. nov.

Ustilago isachnes Syd., Ann. Myc. **12**: 77. 1912.

Ustilago isachnes Saw., Taiwan Agr. Res. Inst. Rept. **87**: 33. 1943.

On *Isachne globosa* (Thunb.) Kuntze, Fengsan, Kaohsiung, coll. Y. Fujikuro (type of *U. isachnes* Saw.), Oct. 28, 1911.

The spores of Sydow's species were originally described as 8–14 × 7–10 μ in size, while those of Sawada's species were 13–18 × 12–14 μ. A comparison of collections from Taiwan and from the Philippines (Herb. Bureau of Science, P. I. No. 4864), however, revealed a close similarity in their spore size, being 10.5–16.5 × 10.5–13.5 μ and 11.5–17.2 × 10.5–13.5 μ respectively. The spores of the Taiwan collection were also more reddish colored than those of the Philippine collection. Notwithstanding the slight variation, these two collections can be reasonably well referred to one species. The presence of a central columella and a pallid false membrane in the sorus indicates that it belongs to *Sphacelotheca*.

33. SPHACELOTHECA REILIANA (Kuehn) Clint., Jour. Myc. **8**: 141. 1902.

On *Sorghum vulgare* Pers., Taitung, coll. K. Sawada, May 22, 1911.

34. SPHACELOTHECA SORGHI (Lk.) Clint., Jour. Myc. **8**: 140. 1902.

Ustilago sorghicola Speg., Anal. Mus. Nac. Buenos Aires III. **1**: 58. 1902; Sawada, Agr. Exp. Sta. Gov't Formosa Spec. Bull. **19**: 335. 1919.

On *Sorghum vulgare* Pers., Taipeh, coll. K. Sawada, Sept. 20, 1908; coll. Y. Fujikuro, June 30, 1910.

35. SPHACELOTHECA TANGLINENSIS (Tracy & Earle) Zundel, Mycologia **36**: 406. 1944.

Ustilago tonglinensis Tracy & Earle, Bull. Torrey Bot. Club **22**: 175. 1895.

Ustilago ischaemi-akoensis Saw., Taiwan Agr. Res. Inst. Rept. **87**: 33. 1943.

On *Ischaemum ciliare* Retz., var. *genuinum* Hack., Taipeh, coll. K. Sawada, Jan. 17, 1944; coll. L. Ling, Apr. 9, 1947; on *Ischaemum aristatum* L. (probably = *I. akoense* Honda), Fêngsan, Kaohsiung, coll. Y. Fujikuro (type of *U. ischaemi-akoensis*), Oct. 28, 1911.

The type of this species was collected in Tanglin, Singapore, and the specific epithet is obviously based on the locality. On the labels of the specimens of the type collection in both the Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture, and the New York Botanical Garden, the specific name and the locality were spelled "tanglinensis" and "Tanglin" respectively. In publication, however, both appeared as "tonglin." It is assumed to be a typographic or unintentional error, and, accordingly, the specific name is corrected here. The following description is based on the type.

Sori in the ovaries, infecting all the florets in the inflorescence, 3–4 mm. long, concealed by the glumes but later protruding, each covered by a pallid false membrane which ruptures at maturity, revealing a dark dusty spore mass surrounding a simple central columella; sterile cells of false membrane subglobose, oval, or angular, chiefly in chains, 7.5–20 × 6.7–13 μ; spores reddish brown, globose, subglobose or ellipsoid, echinulate, 9.5–11.5 μ diam., or 9–12 × 8.7–10 μ.

36. MELANOPSICHIUM PENNSYLVANICUM Hirschh., Notas Mus. La Plata, Bot. **6**: 149. 1941.

On *Polygonum lapathifolium* L., Taipeh, coll. K. Sawada, Aug. 4, 1924; on *Polygonum minus* Hudson ?, Taipeh, coll. Y. Hashioka, Aug. 1934.

37. CINTRACTIA AXICOLA (Berk.) Cornu, Ann. Sci. Nat. Bot. VI. **15**: 279. 1883.

Cintractia fimbristylis-kagiensis Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. **2**: 79. 1922.

Cintractia suedae Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. **2**: 81. 1922.

On *Fimbristylis diphylla* Vahl, Taipeh, coll. H. Sueda (type of *C. suedae*), Nov. 12, 1920; Taichung, coll. Y. Fujikuro, Aug. 6, 1911; Baikei, Taichung, coll. Y. Hashioka, July 3, 1936; Taipeh, coll. Y. Hashioka, Aug. 1934; on *Fimbristylis kagiensis* Hayata, Taichung, coll. Y. Fujikuro (type of *C. fimbristylis-kagiensis*), Oct. 11, 1913.

Cintractia fimbristylis-kagiensis and *C. suedae* were both described as attacking the ovaries. The current taxonomic concept, however, includes such forms under *C. axicola*, though it is very unusual to find in one collection both the florets and peduncles infected. Sawada also noted some difference in spore color between his two species, but it is not shown in the specimens.

38. CINTRACTIA LEUCODERMA (Berk.) P. Henn., Hedwigia **34**: 335. 1895.

Cintractia albida Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 93. 1935.

On *Rynchospora corymbosa* (L.) Britt., Taipeh, coll. T. Suzuki (type of *C. albida*), May 1932.

Ito states that in *Cintractia albida* the warts on the spore membranes are never arranged in parallel lines as are those of *C. leucoderma*, but, to the writer's knowledge, the latter species has never been characterized in that way.

39. CINTRACTIA MINOR (Clint.) Jacks., Mycologia **12**: 153. 1920.

On *Cyperus distans* L., Taichung, coll. Y. Hashioka, July 2, 1936; on *Cyperus compressus* L., Taitung, coll. K. Sawada, May 21, 1911; Sungsan, Taipeh, coll. Y. Hashioka, Aug. 4, 1933, on *Cyperus malaccensis* Roxb., Taipeh, coll. Y. Fujikuro, Sept. 27, 1917.

40. FARYSIA CARICIS-FILICINAE Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 91. 1935.

Farysia pseudocyperi Zundel, Mycologia **23**: 297. 1931.

On *Carex cruciata* Wahl., Taichung, coll. Y. Kudô and S. Sasaki (type), Sept. 17, 1919; Mt. Hsinkao, Chia-i, coll. T. Kawakami, Oct. 14, 1906; Taichung, coll. Y. Hashioka, Nov. 6, 1932.

Ustilago olivacea (DC.) Tul. f. *pseudocyperi* De T. is a name given to a fungus on *Carex pseudo-cyperus* L. from Argentina. Zundel applied this name to a collection on an undetermined member of the Cyperaceae made by F. A. McClure in Kwangtung, China, and transferred it to *Farysia* raising it to specific rank. Both the collections from Argentina and Kwangtung were examined and proved to be different. The Argentina collection (Speg., Dec. Myc. Argent. 3) is *Farysia olivacea* (DC.) Syd.

Ito's description of *Farysia caricis-filicinae* was based on a fungus identical with the Kwangtung collection, but from Taiwan. The hosts of both the Taiwan and Kwangtung collections were determined by Dr. E. H. Walker as *Carex cruciata* Wahl., which is very similar to *Carex filicina* Nees. The name *Farysia pseudocyperi*, although it has priority, was based on an erroneous identification and has been applied to two different organisms. As its application has become a source of confusion, it should be rejected as a *nomen ambiguum* under the provisions of article 62 of the International Rules of Botanical Nomenclature.

F. caricis-filicinae is similar to *Farysia merrillii* (P. Henn.) Syd. in the general appearance of the sorus and the spores. The latter has slightly larger and darker spores with coarser verrucae. The frequency distribution of spore measurements of the three collections concerned are given below:

F. merrillii (type, E. D. Merrill 4915)

Diameter (μ)	8.04	8.71	9.38	10.05	10.72	11.39	12.06	12.73
Frequency	8	23	26	28	11	2	1	1

Mean = 9.55 μ

F. caricis-filicinae (type)

Diameter (μ)	5.36	6.03	6.70	7.37	8.04	8.71	9.38	10.05
Frequency	1	2	24	29	31	6	4	3

Mean = 7.61 μ

F. caricis-filicinae (F. A. McClure, June 10, 1926)

Diameter (μ)	5.36	6.03	6.70	7.37	8.04	8.71	9.38	10.05
Frequency	2	3	5	18	29	22	14	7

Mean = 8.21 μ

41. SOROSPORIUM ANDROPOGONIS-ACICULATI (Petch) Petch, Ann. Roy. Bot. Gard. Peradeniya **5**: 227. 1912.

On *Andropogon aciculatus* Retz., Hsinchu, coll. K. Sawada, June 6, 1917.

42. SOROSPORIUM FLAGELLATUM Syd. & Butl., Ann. Myc. **5**: 489. 1907.

On *Ischaemum timorense* Kunth. var. *peguense* Hack., Taipeh, coll. Y. Fujikuro, Apr. 24, 1911.

43. SOROSPORIUM FORMOSANUM (Saw.) Saw., Dept. Agr. Gov't Res. Inst. Formosa Rept. **35**: 29. 1928. (Fig. 1, b)

Ustilago formosana Saw., Trans. Taiwan Nat. Hist. Soc. no. 34, p. 6. 1918.

Ustilago overceimi Cif., Nuo. Giorn. Bot. Ital. N. S. **40**: 254. 1933.

Sorosporium punctatum Malenç. & Yen, Rev. Myc. N. S. **2**: 130. 1937.

Sorosporium overceimi (Cif.) Malenç., Rev. Myc. N. S. **10**: 121. 1945.

Sori destroying the inflorescence, 4.5–10 cm. long, first each covered by a pale brown false membrane, which gradually flakes away revealing numerous long, slender threads and the dark spore mass; spore-balls rather permanent, 60–195 × 45–115 μ, reniform or broadly elliptical; spores deep brown, finely punctate, 5–7.5 × 4.5–6.5 μ, inner spores lighter in color.

On *Panicum repens* L., Taipeh, coll. Y. Fujikuro (co-type), Apr. 23, 1907; coll. Y. Hashioka, June 1, 1932; coll. K. Sawada, June 21, 1946.

The following species have been recorded on *Panicum repens* with habitat similar to *Sorosporium formosanum*: *Ustilago digitariae* (Kze.) Wint., *U. overceimi*, *Sorosporium punctatum*, *S. tanganyikeanum* Zundel, and *S. yoshinagae* Zundel. The last-named species was not available for study. *S. tanganyikeanum* is

readily distinguishable from the others by the presence in the sorus of very fine shreds of host tissue, measuring up to 250 μ in width. The identity between *S. punctatum* and *U. overeemi* has been discussed by Malençon (**3**). An examination of the type of *S. punctatum* and a specimen on the same host from Java, where *U. overeemi* was originally collected, discloses both in agreement with *S. formosanum*.

The relation between *U. digitariae* and *S. formosanum* deserves special consideration. Several collections on *Digitaria* from Europe and Mexico, labelled as *U. digitariae*, were studied and found to possess rather evanescent spore-balls and smooth spores. On the other hand, collections on *Panicum repens* labelled the same from Algeria, Spain, and Ceylon, in spite of some variation in the spore color, may be classified satisfactorily as *S. formosanum*. The only exception is a collection from India on that host, which has spore-balls rather irregular and less permanent than the others. Nevertheless, the name *Sorosporium digitariae* (Kze.) Padw., as based on the Indian collection on *Panicum repens*, should not be used until authentic specimens on the type host, *i.e.*, *Digitaria sanguinalis* (L.) Heist., are studied.

Besides collections from Taiwan, the following specimens on *Panicum repens* have been included in the present study. MAROC: Rapat, coll. G. Malençon (type of *S. punctatum*), May 1934. JAVA: Buitenzorg, coll. R. D. Rands, Aug. 1920. ALGERIA: coll. L. Ducellia & P. Hariot, 1911 (Vestergr., Myc. Rar. Sel. 1522). SPAIN: Montjnich, Barcelona, coll. E. Gros, Aug. 1918. CEYLON: Hakgala, coll. T. Petch, May 1913. INDIA: Zelwal, Mysore, coll. E. J. Butler, Sept. 9, 1904.

44. SOROSPORIUM PASPALI-THUNBERGII (P. Henn.) Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 94. 1935.

On *Paspalum scrobiculatum* L., Taipeh, coll. K. Sawada, Mar. 14, 1910; also July 8, 1942.

45. **Dermatosorus** Saw., gen nov.

Soris ovaria destruentibus, pseudomembrana atra crassa coriacea prominenti irregulariter dehiscenti tectis; sporarum glomerulis sporis fertilibus numerosis compositis, et cortice pseudoparenchymatico cinctis; sporis e magnitudine media, germinatione ignota.

Species typica: *Dermatosorus eleocharidis* super *Eleocharis dulcis*.

Sori destroying the ovaries, each covered by a firm, thick, dark-colored pseudomembrane which later ruptures irregularly, revealing the mass of spore-balls; spore-ball consisting of numerous fertile spores and a delicate cortex of pseudoparenchymatous tissue; spores medium-sized, germination unknown.

Type species: *Dermatosorus eleocharidis* on *Eleocharis dulcis*.

Dermatosorus eleocharidis Saw., sp. nov. (Fig. 1, c; and 2, d)

Soris in ovariis, saepius antherarum partes inferiores perdentibus, primo membrana falsa crassa nigra coriacea tectis, qua dein rumpit et massam sporarum glomerulorum detegit; cellulis membranae firme conjunctis, flavo-brunneis, ellipsoideis, ovoideis vel angularibus, 9–16 × 6.5–15 μ, pariete 1.5 μ crasso; sporarum glomerulis sporis numerosis compositis, rectangularibus, ellipticis vel subglobosis, opacis, 112–606 × 88–358 μ diam., cortice delicato sed subpermanenti, pseudoparenchymatico, atro, 15–75 μ crasso cinctis, cellulis corticis sterilibus, angularibus, rufo-brunneis, 3–6.5 μ diam.; sporis laxe connexis, globosis usque ellipsoideis, 6–11 μ diam., praecipue 7–9 μ, episporio rufo-brunneo, 1 μ crasso, levi.

In ovariis *Eleocharidis dulcis* Trin.

Sori in the ovaries, often involving the basal parts of anthers, glumes intact, at first each covered by a firm, thick, black, false membrane, which is composed of yellow brown cells, firmly bound together, ellipsoidal, ovoid or angular in outline, 9–16 × 6.5–15 μ in diameter, with walls 1.5 μ thick, and which later rupture irregularly, revealing an aggregate of spore-balls; spore-balls many spored, rectangular, ellipsoidal or subglobose, opaque, 112–606 × 88–358 μ diam., covered by a delicate but rather permanent cortex of pseudoparenchymatous tissue, 15–75 μ thick, consisting of reddish brown cells, 3–6.5 μ diam., with walls 1 μ thick; spores loosely united, globose to ellipsoidal, 6–11 μ diam., chiefly 7–9 μ, epispore smooth, reddish brown, approximately 1 μ thick.

On *Eleocharis dulcis* Trin., Kangsan, Kaohsiung, coll. Y. Shimada (*type*), April 10, 1919.

As the germination of the spores is not known, the taxonomic position of this genus is uncertain. According to the structure of the sori and the spore-balls, it probably belongs to the Ustilaginaceae and is closely related to *Sorosporium*, from which it differs in the presence of a cortex layer covering the spore-ball.

46. TOLYPOSPORIUM BULLATUM (Schroet.) Schroet., Krypt. Fl. Schles. **3**: 276. 1887.

On *Echinochloa crus-galli* Beauv., Taipeh, coll. K. Sawada, Nov. 5, 1927.

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A NEW OLPIDIOPSIS PARASITE OF KARLINGIA ROSEA FROM MARYLAND

JOHN S. KARLING[1]

(WITH 17 FIGURES)

This parasite occurred in cultures of *K. rosea* which had been isolated and grown on bits of onion skin in a water and soil sample collected from a stagnant pool in a pig pen near Frederick, Maryland in April, 1948. Within two weeks after the host sporangia had developed in the substratum a large number of them were found to be infected, and in the course of a month the disease had reached epidemic proportions in several of the cultures. In such cultures as high as fifty-two per cent of the sporangia were parasitized, but in others infection was less severe and even lacking. The epidemic began to subside in six weeks, and after two months no trace of the parasite, except its resting spores, could be found.

As far as present studies go, this fungus appears to be an obligate parasite of *K. rosea*. All attempts to infect cultures of *Karlingia* sp., *K. hyalina, K. granulata, Chytriomyces hyalinus, C. aureus, C. stellatus, Asterophlyctis sarcoptoides, Phlyctorhiza variabilis, Nowakowskiella elegans, Septochytrium variable,* and *Cladochytrium replicatum* have failed.

This parasite is characterized by a monocentric, holocarpic thallus; spherical to oval, pyriform, obpyriform, oblong, or slightly angular sporangia; biflagellate, heterocont, reniform zoospores, and asexual, spherical, oval, oblong or angular resting spores. Morphologically and developmentally its thallus, sporangia and zoospores resemble those of *Olpidiopsis* species, but it differs from most members of this genus by its asexual resting spores. How-

[1] This work was begun at the Chesapeake Biological Laboratory, Solomons, Md., where the author was guest investigator in the spring and early summer of 1948. The author is indebted to the Department of Education and Research, State of Maryland, and particularly to the Director of the Laboratory, Dr. R. V. Truitt, for providing research funds and laboratory facilities for this work.

ever, if this genus is interpreted to include species with parthenogenetic or asexual resting spores, as the author (**1942**) has done, the present parasite belongs in *Olpidiopsis*.

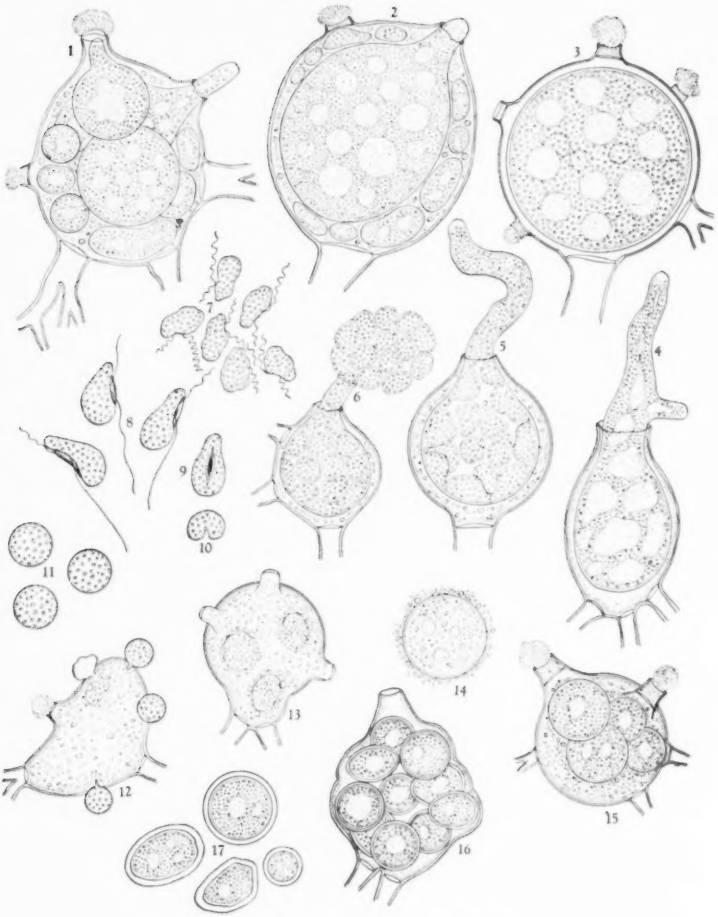
With the exception of the doubtful *Pseudolpidium* (*Olpidiopsis*) *Sphaeritae*, this is the only species of the family Olpidiopsidaceae known to parasitize a chytrid, and since it appears to be an obligate parasite of *K. rosea* and differs morphologically from other species of *Olpidiopsis* it is regarded as a new species and named *O. Karlingiae* after its host.

Olpidiopsis Karlingiae sp. nov. Sporangiis solitariis vel numerosis, levibus hyalinis, sphaericis 8–120 μ, ovalibus 15–84 × 20–98 μ, oblongatis, 6–10 × 12–28 μ, pyriformibus, obpyriformibus, aut angularibus, tubulis exeuntibus 6–12 × 14–18 μ aut 7 × 110 μ. Zoosporis hyalinis minutissime granulatis, reniformibus 6–6.5 × 10–10.8 μ, cum duobus nonaequalibus flagellis 6.5–7 μ et 11.6–13 μ longo. Sporis perdurantibus hyalinis, levibus, sphaericis 8–22 μ, ovalibus, 9–17 × 15–23 μ, oblongatis, 7–9 × 12–20 μ, aut angularibus; germinatione ignota.

Sporangia hyaline, smooth, spherical, 8–120 μ, oval, 15–84 × 20–98 μ, oblong and slightly curved, 6–10 × 12–28 μ, pyriform, obpyriform or slightly angular when pressed together, with one simple, rarely two and branched, short and broad, 6–12 by 14–18 μ, or long, 7 × 110 μ, discharge tubes which protrude directly through the host cell wall or the exit papillae. Zoospores reniform, 6–6.5 × 10–10.8 μ, and containing 30 to 50 minute granules which are not particularly refractive; heterocont, anterior and posterior flagella 6.5–7 μ and 11.6–13 μ long, respectively. Resting spores spherical, 8–22 μ, oval, 9–17 × 15–23 μ, oblong, 7–9 × 12–20 μ or angular when pressed together, with a hyaline, smooth, 1.8–2.2 μ, thick wall, evenly but coarsely granular protoplasm and one or more vacuoles; germination unknown.

Parasitic in *Karlingia rosea*, Frederick, Maryland.

In general appearance this species is strikingly similar to *Olpidium Rhizophlyctidis* recently described by Sparrow (**1948**) as a parasite of a variant of the same host, and the two species might be easily mistaken until the zoospores are examined and compared. As shown in figures 1–3, one or several parasites develop in a host sporangium, and in exceptional cases as many as fifty-six have been observed in one host cell. In such instances the size of the parasite is relatively small, depending on the number present. In cases of single infection the subsequent thallus may almost fill the



FIGS. 1–17. *Olpidiopsis Karlingiae.*

host sporangium (FIG. 3). When multiple infection occurs one or more of the thalli may become relatively large and crowd the smaller ones towards the periphery (FIGS. 1, 2). If several thalli develop equally in size and become large, the resulting sporangia may be distinctly angular in outline from mutual contact and pressure.

The effect of the parasite on the host is fairly constant. No marked enlargement of the sporangia occurs, and only occasionally

are they malformed or distorted. This may occur in cases of multiple infection as the parasites enlarge and cause local bulging of the sporangium wall (FIG. 16). So far no physical antagonism between the host and parasite protoplasm has been observed. The host protoplasm usually aggregates in a dense layer around the parasites (FIGS. 13, 14) without any marked zone of antagonism, as in other species of *Olpidiopsis*. As the parasites enlarge, the host protoplasm is largely absorbed, and finally only a residual layer remains around the mature parasites (FIGS. 1, 2, 5, 6, 15). However, in two cases of single infection only a portion of the host protoplasm was absorbed, while the remainder underwent cleavage and formed normal zoospores.

The method of infection and development of the parasite within the host are fundamentally similar to those of other *Olpidiopsis* species. As shown in figure 12 the zoospore comes to rest on the host cell and forms a short germ or penetration tube through which its content passes into the host protoplasm. The empty zoospore case soon becomes wrinkled, collapses and disappears. The newly-entered parasite is difficult to see in the pink or rosy host protoplasm, and it is frequently completely obscured. However, as it grows and increases in size it becomes more clearly distinguishable with a well-defined wall, which may often be surrounded by a layer of rosy or reddish, refringent globules from the host protoplasm (FIG. 13). In the young stages the protoplasm of the parasite has a grayish refractive appearance like that of other species of *Olpidiopsis* and usually includes several refractive bodies. As it increases in size small vacuoles appear in the cytoplasm (FIG. 14), and with continued growth and development these increase in number and size (FIGS. 3, 4). Eventually and usually they coalesce to form one large irregular central vacuole, but in some thalli they may remain relatively small and numerous and retain their entity after cleavage has been completed (FIG. 2) as Barrett (1912) described for *O. vexans*. By this time the protoplasm of the parasite has an even but coarsely, grayish granular appearance (FIGS. 1–4), and the refractive material appears to be highly dispersed. The incipient discharge tube begins as a low broad papilla which then elongates and usually grows out through an exit papilla of the host (FIGS. 4–6). Occasionally, it may perforate and push through the host

wall directly (FIG. 2). As noted elsewhere, it may be short and broad (FIGS. 2, 6) or elongate, curved, rarely branched (FIG. 5), and tapered toward the apex. In mature sporangia with a large central vacuole, the protoplasm usually forms a scalloped layer around the vacuole. Broad V-shaped cleavage furrows form at the border of the vacuole (FIG. 5), progress to the periphery, and thus delimit the zoospore initials. The cleavage segments are well defined at first, but soon become obscured as they increase in size. Shortly after this stage the tip of the discharge tube deliquesces and the zoospore initials flow out (FIG. 6) and form a globular quiescent mass. After a short while they separate and start rocking back and forth as the flagella begin to beat. At this stage the zoospores are somewhat irregular in shape (FIG. 7) without easily recognizable anterior and posterior ends and ventral grooves, but as the rocking continues they mature and assume definitive shape and organization. The rocking movement becomes increasingly rapid, and within 2 to 3 minutes the zoospores swim away. So far no swarming of the zoospores in a vesicle outside of the sporangia has been observed. However, discharge and maturation of the zoospore initials has been seen only a few times, and it is possible that the process and behavior described above may be exceptional instead of general.

The mature zoospore is reniform with a tapering anterior and a rounded posterior end (FIG. 8) and contains from 30 to 50 granules. In the latter respects they resemble very closely those of *Lagenidium Distylae, L. humanum* (Karling, **1944, 1947**) and species of *Pythium*. The two unequal flagella are inserted in the ventral groove and extend in opposite directions. In swimming the zoospore rotates slowly on one axis, and the anterior flagellum beats more rapidly than the posterior one, which often appears to be dragged along behind. After swimming about for one-half to three-fourths of an hour, the zoospores slow up, come to rest, retract their flagella and round up (FIG. 11). So far no evidence of diplanetism has been observed, and if the zoospores do not come to rest on a host cell and germinate they degenerate within a few minutes.

As noted previously, the resting spores are apparently formed asexually. No evidence of fusion between so-called male and fe-

male thalli has been found. Up to a certain stage the development of the incipient resting spore is very similar to that of the sporangia, and the two are difficult to distinguish in the young stages. However, the wall of the incipient spore soon begins to thicken, and the content becomes more densely granular (FIG. 16). At the same time the small vacuoles usually coalesce and form a larger central one (FIGS. 15, 16) which may be irregular or spherical in shape and up to 10 μ in diameter in exceptional cases. On the other hand, it is not uncommon to find mature spores with 2 or 3 small vacuoles (FIG. 17). Like the sporangia, the resting spores may occur singly or in large numbers in a host cell (FIG. 16). In rare cases as many as 43 were found in one host sporangium, which was distorted by the tightly packed spores. So far germination of the resting spores has not been observed.

In connection with the description of this new species it may be noted that other related members of the Olpidiopsidaceae were found and isolated in soil and water collections from various parts of Maryland. These are: *Pythiella vernalis* (Couch, **1935**) parasitizing *Pythium* sp. in filaments of *Vaucheria germinata* from a pond near Long Beach, Calvert County; *Olpidiopsis Aphanomycis* parasitic in *A. laevis*, Wicomico River, Charles County and near Centerville, Queen Anne's County; *O. Pythii* in *Pythium* sp., near Centerville, Queen Anne's County; and *O. Achlyae* in *Achlya flagellata*, Old Spout Farm, Calvert County.

In addition to these members of the Olpidiopsidaceae, three species of the family Lagenidiaceae were found and isolated: *Lagenidium pygmaeum* in apple pollen, Solomons Island, Calvert County; *L. Closterii* in *Closterium setiferum* from a pond near Long Beach, Calvert County, and *L. humanum* in dead human skin, Old Spout Farm, Calvert County.

SUMMARY

Olpidiopsis Karlingiae is an endobiotic parasite of the sporangia of the chytrid *Karlingia rosea*. It developed in a culture of this host which had been isolated on bits of onion skin from a soil and water sample near Frederick, Maryland in April, 1948. It is characterized by a monocentric holocarpic thallus, spherical, oval, pyriform or oblong sporangia, with a broad, short or long discharge

tube, reniform, biflagellate, heterocont zoospores, and asexual, spherical, oval, oblong or angular resting spores. So far it appears to be an obligate parasite of *K. rosea* since all attempts to infect eleven other chytrids have failed.

In addition to this species seven other related simple holocarpic biflagellate Phycomycetes were isolated from water and soil in various parts of Maryland.

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EXPLANATION OF FIGURES

FIG. 1. Sporangium of *Karlingia rosea* parasitized by 3 large and 6 small thalli of *O. Karlingiae*. FIG. 2. Same host with one large and ten small parasites. FIG. 3. Same host with a single large parasite. FIG. 4. Sporangium of *O. Karlingiae* with numerous irregular vacuoles and a broad, tapering, branched discharge tube. FIG. 5. Centrifugal cleavage in a sporangium with a long curved discharge tube. FIG. 6. Discharge of zoospores. FIG. 7. Maturing zoospores undergoing rocking movements. FIG. 8. Side views of zoospores showing heterocont flagella and ventral groove. FIGS. 9, 10. Ventral and cross section views of zoospores. FIG. 11. Zoospores after coming to rest and rounding up. FIG. 12. Infection stages. FIG. 13. Young parasites within host protoplasm. FIG. 14. Later developmental stage. FIG. 15. Host sporangium with four incipient resting spores. FIG. 16. Distorted host sporangium with ten mature resting spores. FIG. 17. Variations in size and shape of mature resting spores.

CONCERNING THE IDENTITY OF ITERSON'S CELLULOLYTIC MYCOGONE

WILLIAM W. DIEHL

In various published discussions of the role of fungi in relation to aerobic deterioration of cellulosic materials there are references to one of the two pioneer research papers by the Dutch microbiologist, Iterson (**1, 2**), in which among a list of fifteen species of fungi the most active one was reported under the name *Mycogone puccinioides* (Preuss) Sacc. (syn. *Blastotrichum puccinioides* Preuss). Since Iterson's account was published this name has been quoted liberally in various articles concerned with deterioration of fabrics and other cellulosic materials, notably in the well-known compendium by Thaysen and Bunker (**3**). In that compendium the brief description of *Blastotrichum puccinioides* agrees, unfortunately, with the original description of that species by Preuss (**4**) rather than with Iterson's records. Preuss's *Blastotrichum puccinioides* was described and illustrated as having septate spores from a mycelium growing on a mushroom and was distinctly different from the amerosporous organism so identified by Iterson (l.c.). A review of the literature of cellulolytic deterioration suggests the likelihood that specific agents variously referred also to species of the form-genera *Acremonium, Acremoniella, Sepedonium, Monotospora,* etc., may well be closely related, if not the same, species as that reported by Iterson, but it is convenient to restrict the present discussion to the identity of Iterson's oft-cited fungus.

Among the cultures frequently obtained from decaying cotton fabrics is a dark-spored hyphomycete which has features suggesting the likelihood of its identity with Iterson's fungus. I first isolated it in pure culture in 1945 from a cotton fabric which had recently suffered decay through use in military operations in the South Pacific area. It was isolated readily using a modification of Iterson's (l.c.) technique in recovering the organism which grew most rapidly following the placing of a bit of the decayed fabric as inoculum upon sterile filter paper lying on a petri-dish plate of sterile

mineral salts agar. With filter paper as the sole source of carbon the black-spored fungus that grew over the filter paper was obviously the active agent in its decomposition. This same fungus had previously been encountered and isolated by P. B. Marsh and Katharina Bollenbacher in the course of studies of the etiology of fabric deterioration. They had isolated it from fabrics after exposure to the elements in various parts of the world and have since recovered it also from cotton fabrics that had been buried experimentally in native soils at Beltsville, Maryland. These isolates, although showing much variation among themselves and incidentally much given to sectoring, appeared enough alike to be considered one species, which is here identified as *Humicola fusco-atra* Traaen (**5**). This species was originally described from a culture isolated from soil in Norway and has since been recorded from the U. S. by Mason (**6**) after examination of a culture sent to him by Dr. S. A. Waksman as an isolate from soil in New Jersey. Most curiously this specific name appears to have been accorded little or no attention by workers concerned either with soil microbiology or with problems of cellulolytic deterioration; if the species was encountered, as seems likely, it has been reported under different names. It seems especially odd also that no consideration has been given to Iterson's fungus under the name *Humicola* since Traaen (l.c.) himself suggested that it might be the same as his species *H. grisea*. It appears to me, however, that Traaen's suggestion is confusing because the spore dimensions of his *H. grisea*, "12–17 μ," are very different from those that may be calculated from Iterson's illustrations of his cellulolytic fungus. By measuring the spores as shown by Iterson and calculating their sizes in the light of the stated magnification they measure 7.25–8.18 μ, which is well within the dimensions "6–9–12 μ" as given by Traaen for his *H. fusco-atra*. It is, furthermore, pertinent to note that the cultures of cellulolytic fungi isolated by Dr. Marsh and his associates and by myself, which I have referred to *H. fusco-atra*, are very similar to the culture of *H. fusco-atra* kindly furnished me in 1945 by Dr. S. A. Waksman; this was a transfer from the same original Waksman isolate reported as *H. fusco-atra* by Mason (l.c.) after his comparison of it with Traaen's original type culture.

In addition to its practical bearing on studies of deterioration of cellulosic materials and of soil microbiology this species is of concern nomenclatorially. Mason (l.c.), who has given critical consideration to the two different form-genera *Monotospora* of Saccardo and *Monotospora* of Corda, considered Traaen's *H. grisea* in the former as a synonym of *M. daleae* Mason and *H. fusco-atra* as referable to the latter. He pointed out that, if *Monotospora* of Saccardo should be conserved against Corda's prior name, *Humicola* as a synonym of the discarded *Monotospora* of Corda would then be legitimate with *H. fusco-atra* Traaen as the type species. Because of its convenience this should be a welcome taxonomic disposition of that hitherto debated form-genus.

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COLUS SCHELLENBERGIAE AGAIN

W. C. Coker and G. C. Rebell

(with 1 figure)

Two previous collections of *Colus Schellenbergiae* Sumstine (**7**), now generally considered as probably identical with *C. javanicus* Penzig (**4**), have been reported for North America: the type, Pittsburgh, Pa., and one by Dr. Seaver in the New York Botanical Garden (**5**). The American plant was first illustrated by Dr. Seaver (**6**, pl. 8) and has not been illustrated since.

The present report concerns two additional collections, as follows.

Miss Jane Miller (now Mrs. Rebell), Summit, New Jersey, July 27, 1938 (Univ. N. C. Herb.). Fourteen sporophores appeared and matured in a few days on a rotted log toward the end of an excessively rainy summer. All sporophores had three arms roundly arched and united at the tips. None were found there the following year or since.

G. C. Rebell, Ridley Park, Pa., Aug. 4, 1948. More than seventy-four sporophores appeared in woods during rainy weather throughout August; growing from rotted wood and humus in an area about 50 by 100 feet, in part marked by the site of a former ornamental garden. A description of these plants follows.

Volva spherical to ovoid, 1–1.7 cm. in diameter, white or dirty brown; stalk an open cup or tube (depending on the length), 0.6–3.7 cm. long, 0.5–1.1 cm. wide at center when flattened, flaring out at the top to support the arms. Arms 3 (in one plant, 2; three plants in Seaver's illustration show four arms), hollow, 1.7–6.8 cm. long, 0.3–0.9 cm. thick at base, tapering upward to attenuated tips that are loosely united and tend to separate after expansion if much disturbed; surface of arms crumpled and pitted, orange-red, paler at base. Gleba dark green, strongly fetid, attached to the inner faces of the arms from the tips to near the base. Spores hyaline, ellipsoid, 1.5–2 × 4–5(5.5) μ.

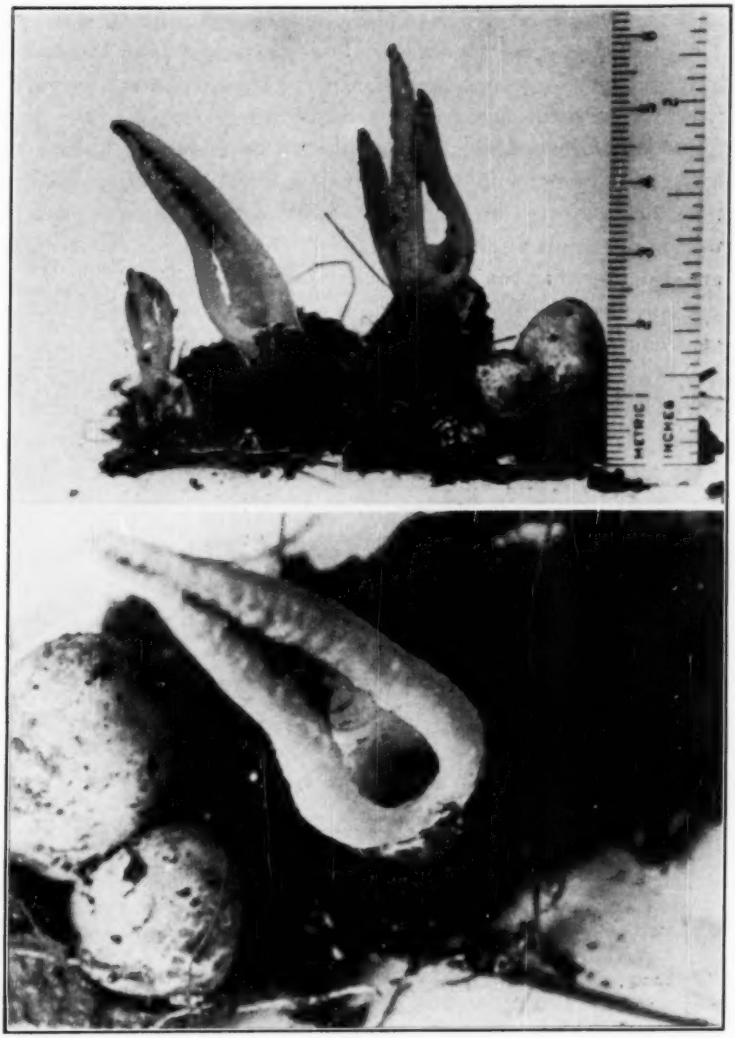

FIG. 1. *Colus Schellenbergiae*. Ridley Park, Pa. Lower figure × 2½. Photographs by G. C. Rebell.

Like other Phallaceae, the plants tend to vary greatly in size, those developing on dry days being smaller and paler than those developing under wet conditions. Our measurements of 16 plants in fresh condition gave a height, counting stalk and arms, varying from 3.3 to 9.6 cm. as extremes, most of them about 4.6 to 6.4 cm. Sporophores developing in wet weather in the Ridley Park site are larger than those found by Miss Miller in 1938, but mostly smaller than those reported by Sumstine. Most of the stalks are shorter than in the plants illustrated by Seaver. Our spore measurements are narrower than the figures given by Sumstine.

Lloyd's genus *Pseudocolus* (**3**), it seems to us, may well be recognized for those species which have a stalk with simple arms tapering toward, and united at, the tips. The name would then be *Pseudocolus Schellenbergiae* (Sums.) Johnson (**2**). The original *Colus* (**1**) consists of a stalk with arms that form a small latticework at the top. There are several other species with characters similar to the present one, and it would seem convenient to recognize them as a definite group under the genus *Pseudocolus*.

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NOTES ON SOME SPECIES OF PHAKOPSORA AND ANGIOPSORA

M. J. Thirumalachar and Frank D. Kern

(with 3 figures)

In the course of a cursory study of some species of *Phakopsora* deposited in the Pennsylvania State College herbarium, interesting features were noticed in some of the species which warranted a re-study. Comparative studies of the related genera of *Phakopsora* were made in order to have a better knowledge of the morphology of the spore forms for improving our taxonomic concepts of the group.

The genus *Phakopsora* Dietel (**1895**) was based on *Phakopsora punctiformis* on *Galium aparine* collected in India by Barclay. Dietel (**1890**), calling the rust a *Melampsora*, emphasized the fact that the teliospore crusts had spores which were not arranged in chains, a condition brought about by younger teliospores developing and pushing into the sorus between older ones (fig. 3). While this feature of teliospore production is accepted by us, and by most uredinologists, the need for confirmation by examination of the type of the genus still remains. An attempt to locate the type specimen at Kew Herbarium has been unsuccessful. The mode of spore development in chains, or in irregular succession, has been used recently in separating rust genera. Some of the results of studies on the *Phakopsora* species are presented here.

Phakopsora dominicana Kern (**1928**) was described as a microcyclic rust-species on *Croton angustatus*. The material was collected in Santo Domingo by Dr. C. E. Chardon. The telia are formed on slightly hypertrophied spots, black, and densely crowded. A careful re-study of the material indicated that the sori are deep-seated developing chains of one-celled, golden-brown teliospores in succession. The telial chains are compactly grouped showing some lateral coalescence, but can be separated by manipulation. The developing chains of spores rupture through the epidermis so that the

sori are erumpent. The mature spores germinate away at the apex (FIG. 1).

The characters enumerated above reveal that the rust is a species of *Bacodromus,* so far known as a microcyclic rust genus on Compositae. The absence of non-erumpent, lenticular telia, with teliospores developing in irregular succession, differentiates it from

FIG. 1. Erumpent telium of *Bacodromus dominicana* on *Croton;* note that the spores are in chains, and that the sorus is deep seated and protruding.

Phakopsora. The rust is transferred under *Bacodromus* as **B. dominicana** (Kern) n. comb.

Chains of one-celled teliospores also occur in *Angiopsora, Arthuria, Dictelia, Cerotelium* and others. Species of *Angiopsora* and *Arthuria* have been mistaken several times for *Phakopsora* or *Dasturella* by workers on rusts. A brief account of their distinguishing characters will be taken up later.

A rust on *Randia armata* from Venezuela was described by

Kern and Thurston (**1944**) as *Phakopsora Randiae*. The uredia are hypophyllous with characteristic incurved paraphyses. The telia are hypophyllous, with 2–6 superposed teliospores forming compact lenticular, non-erumpent crusts (FIG. 2). The spores measure 16–35 × 10–15 μ with apical thickening of 3–7 μ.

A re-study of this rust and comparisons with the descriptions of *Phakopsora melaena* described by Sydow (**1939**) from Ecuador

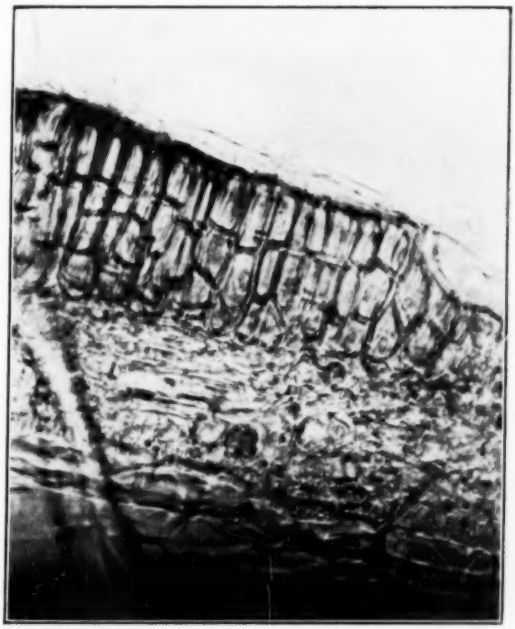

FIG. 2. Indehiscent telium of *Angiopsora melaena* on *Randia* showing teliospores in chains.

revealed that the two rusts are identical and parasitized the same host species. The teliospore measurements given by Sydow are 16–32 × 14–20 μ with the apical thickening of 3–9 μ. The telia are non-erumpent, lenticular; teliospores arranged in chains (FIG. 2). This is a distinguishing character of *Angiopsora* Mains, which differs from *Phakopsora* in the mode of teliospore development. They do not occur in chains in *Phakopsora*, but develop in irregu-

lar succession, the younger spores wedging in between older ones (FIG. 3). This character of chain formation in *Angiopsora* can be clearly seen when spores are of large size, and there are not several spores in a chain. In *A. zeae*, *A. lenticularis*, and others, the chains are very obvious, but less distinct or obscure in some others, where also they do occur in catenulations. The thinness and plane of sectioning are also of importance in determining the teliospore chains. The rust on *Randia armata* is an *Angiopsora* on account of the occurrence of teliospore chains. Since Sydow's *P. melaena*

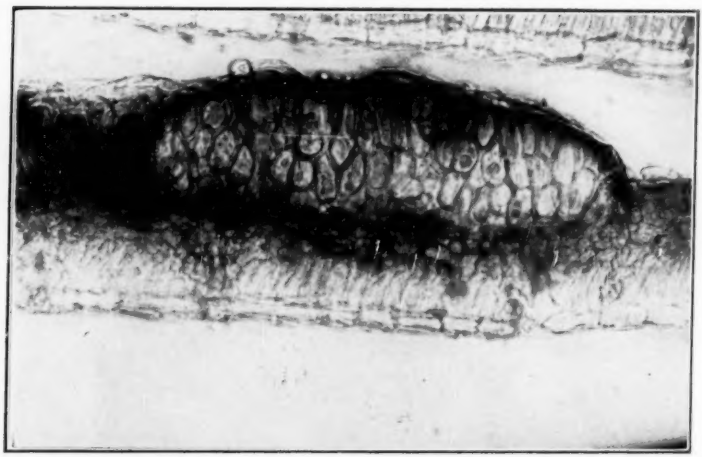

FIG. 3. Lenticular telium of *Phakopsora antiguensis* on *Acalypha* showing the spores in irregular succession, the younger spores developing between the older ones.

was described earlier we propose the following transfer: **Angiopsora melaena** (Sydow) n. comb. (*Phakopsora melaena* Sydow, *P. Randiae* K. and T.)

Examination of the type material of *Phakopsora Hansfordii* Cumm. (**1943**) on *Alcornea cordifolia* shows that it is also a species of *Angiopsora*. The teliospores are oblong-cuboid, and are in chains of 2–6 spores. The excellent illustration given by Cummins (**1943**) brings out this character very well. The rust is transferred under *Angiopsora* as **A. Hansfordii** (Cumm.) n. comb.

Phakopsora venezuelana was described by Sydow (**1930**) on *Sickingia erythroxylonis*. Only uredia were described at first (**1930**) and later Sydow (**1935**) found the telial stages in the same collection. The telia are subepidermal, amphigenous, with 2–8 superposed layers of teliospores. Careful examination of telia of *P. venezuelana* revealed that the teliospores are superposed in chains and following our present concept of separating *Angiopsora* from *Phakopsora*, the rust should be placed under *Angiopsora* as **A. venezuelana** (Sydow) n. comb.

Physopella Burserae was described by Sydow (**1925**) as a leaf rust on *Bursera gummifera* in Costa Rica. The rust was described only in the uredial stage, but taking into account the characteristic incurved paraphyses surrounding the sorus and enclosing sessile urediospores, Sydow placed it under *Physopella*. The genus *Physopella* has been merged with *Cerotelium* or with *Phakopsora* in the absence of telial structures. Several of the species of *Physopella* based upon uredial stages have proved to be *Phakopsora* when the telial stages have been found. *Physopella* (= *Cerotelium*) *desmium* and *P. Cherimoliae*, for instance, have been shown to be species of *Phakopsora* by Cummins.

A careful examination of specimens of *Physopella Burserae* distributed by Sydow (Fungi exotici exsiccati 600, which bears the same date and place of collection as the type specimen) reveals the occurrence of telia. The telia appear microscopically as small brownish-black specks distributed among the uredia. In sections they are lenticular, non-erumpent, with golden-brown teliospores developing in irregular succession and not in chains. Since the telial stage has been discovered, the following change in nomenclature is proposed, **Phakopsora Burserae** (Sydow) n. comb.

Phakopsora Meliosmae was described by Kusano (**1904**) on the leaves of *Meliosma myrianthae* from Japan. The telia were said to be covered by the epidermis, with 3–4 superposed, polyhedral spores which were thin-walled and light brown. An authentic specimen of *Phakopsora Meliosmae* collected by Kusano, and distributed in "Vestergren's Micromycetes Rarius selecti No. 1558," was carefully sectioned and examined. The uredia are hypophyllous and possess the incurved paraphyses of the *Phakopsora*-type.

The telia are non-erumpent, lenticular, and the teliospores are cuboid and in distinct chains. The rust is a species of *Angiopsora* and the combination **A. Meliosmae** (Kusano) n. comb. is proposed.

A leaf rust on *Ampelopsis heterophylla*, collected by Shirai near Tokyo, was described by Dietel (**1898**) as *Phakopsora Ampelopsidis* Dietel & Sydow. In the next year P. Sydow (**1899**) described another species *Phakopsora vitis* on *Parthenocissus Thunbergii*. The urediospores were stated to be more elongated, and the teliospores larger, in *P. vitis* than in *P. Ampelopsidis*. Naoharu Hiratsuka (**1900**) made a comparative study of the two and found them to be identical. He therefore reduced *P. vitis* as a synonym of *P. Ampelopsidis*. This disposition was maintained in later studies on the *Phakopsora* by Naohide Hiratsuka of Japan (**1935**). A collection of the rust on *Ampelopsis heterophylla* collected in Japan in 1929, and distributed as *Phakopsora Vitis* (Thüm.) Sydow, has been available for our study.

The uredia are sub-epidermal, erumpent, encircled by incurved paraphyses and open by a pore at the top. The telia are numerous, appearing as brownish-black specks. They are non-erumpent, lenticular, with cuboid to globular teliospores occurring in definite chains. The catenulations of the teliospores are well illustrated by the Sydows (Monographia Uredinearum **3**: 415, Tab. XVII, FIG. 153. 1914). The rust on *Ampelopsis heterophylla* is an *Angiopsora* according to our present concept of the genus, and is transferred as **Angiopsora Ampelopsidis** (Dietel & Sydow) n. comb. *Phakopsora Vitis* Sydow is regarded as a synonym.

Studies of *Phakopsora* and related genera indicate that there is some confusion in separating the genera. Species described as *Phakopsora* have been placed later under *Angiopsora*, *Arthuria* and *Bacodromus*. *Dasturella* was based upon *Angiopsora divina* Syd. Rust species with only uredial stages have been placed under *Cerotelium* or *Phakopsora* on the basis of presence of hyphoid peridium and paraphyses in the sorus.

A concise key which may help in stressing the important characters already enumerated in literature will not be out of place.

KEY

A. Teliospore walls firm, colored, golden-brown to chestnut-brown: teliospores resting.
  (a) Telia indehiscent, lenticular.
    (1) Teliospores in chains.....*Angiopsora*
    (2) Teliospores developing in irregular succession, younger spores alternating with older ones, wedging to form a compact crust.
      (i) Uredinia opening by a pore-like cavity, with encircling paraphyses developing from hyphoid peridium....*Phakopsora*
      (ii) Uredinia without peridium or paraphyses.....*Bubakia*
  (b) Telia erumpent, protruding, teliospores in chains.
    (1) Telia deep seated.....*Bacodromus*
    (2) Telia in large flabelliform crusts.....*Dasturella*
B. Teliospore walls thin, hyaline; teliospores germinating at maturity.
  (a) Urediospores in chains.....*Arthuria*
  (b) Uredia with hyphoid paraphyses; spores borne singly....*Cerotelium*

The genus *Angiopsora* has usually been considered as being restricted to grass hosts. In these studies we have considered eight species (notes on all species have not been included in this paper) as follows:

A. Ampelopsidis (Diet. & Sydow) n. comb. on *Ampelopsis heterophylla.*
A. compressa (Arth. & Holw.) Mains on *Paspalum* sp.
A. Hansfordii (Cumm.) n. comb. on *Alcornea cordifolia.*
A. lenticularis Mains on *Lasiacis* sp. (type).
A. melaena (Sydow) n. comb. on *Randia armata* (FIG. 2).
A. Meliosmae (Kusano) n. comb. on *Meliosma myriantha.*
A. pallescens (Arth.) Mains on *Tripsacum* sp.
A. phakopsoroides (Arth. & Mains) Mains on *Olyria* sp.
A. venezuelana (Sydow) n. comb. on *Sickingia erythroxylonis.*
A. Zeae Mains on *Zea Mays.*

We do not have a complete list of the species of *Phakopsora.* The following have been examined by us and are placed here with confidence:

P. antiguensis (Cumm.) K. & T. on *Acalypha* sp. (FIG. 3).
P. Burserae (Sydow) n. comb. on *Bursera gummifera.*
P. Cherimoliae (Lagerh.) Cumm. on *Anona* sp.
P. desmium (B. & Br.) Cumm. on *Gossypium* sp.
P. fenestrala Arth. on *Phyllanthus* sp.
P. jatrophicola (Arth.) Cumm. on *Jatropha* sp. and *Manihot* sp.
P. tecta Jackson & Holway on *Commelina* sp.

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PIGMENT PRODUCTION IN THE DIFFERENTIATION OF TRICHOPHYTON MENTAGROPHYTES AND TRICHOPHYTON RUBRUM

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The two most common fungi concerned in the etiology of dermatophytosis of the feet are *Trichophyton mentagrophytes* (*gypseum*) and *Trichophyton rubrum*. The frequency with which these two species occur was shown by Hopkins *et al.* (**1**) in a comparative compilation of figures obtained by different workers. Out of 1,700 cultures, 47 per cent were identified as *Trichophyton gypseum* (*mentagrophytes*) and 36 per cent as *Trichophyton rubrum*. Ariyevich and Pentkovskaya (**2**) reported that the organism most frequently causing epidermophytosis of the feet in U.S.S.R. was, as in most European countries, *Epidermophyton Kaufmann-Wolf* (*Trichophyton gypseum*). *Trichophyton gypseum* (*mentagrophytes*) comprised 94.5 per cent, and *Trichophyton rubrum* 3.2 per cent of 533 cultures they obtained. *Trichophyton rubrum* was the preponderant causative organism isolated by Mu and Kurotchkin (**3**) from cases of tinea of the feet, hands, nails and body observed in Peiping, China. Out of 134 cultures gathered, 110 were determined as *Trichophyton rubrum*.

It can thus be seen that in the routine work of a dermatomycologic clinic where one of the largest groups of patients are those afflicted with dermatophytosis, the problem of species differentiation of *T. gypseum* and *T. rubrum* is frequently encountered. The desirability of this differentiation has been emphasized in the light of prognostic and therapeutic considerations. In typical cases, *T. gypseum* infections run a more acute inflammatory course and are amenable to treatment while those of *T. rubrum* are of chronic, mildly inflammatory character and resistant to medication. Lewis,

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Montgomery and Hopper (**4**) considered the chronic nature, dull red color, slight branny scaling and presence of concomitant lesions as suggestive of *T. rubrum* infections while a short, rapid course marked by dyshidrosis and more acute inflammatory reaction is suspicious of *T. gypseum* infection. Diagnosis based on the above clinical criteria, however, cannot be made with certainty as at not a few times, the same kind of lesion may be produced by different fungi and the same fungus may cause several types of lesions. Hopkins *et al.* (**1**), although agreeing to some extent with the differential points given by Lewis and co-workers, concluded from their experiences that the differences are essentially inconstant.

The final proof for the differentiation of the two types of infection lies in the isolation and identification of the causative organism. At present, the diagnosis is based mainly on the colony characteristics and on morphologic studies in culture mounts. One of us (R. W. B.) (**5**) listed the distinguishing features as:

Trichophyton mentagrophytes (gypseum)	*Trichophyton rubrum*
Honey agar:	
Rapid growth; flat and spreading. Granular to downy surface. White to cream in color. On reverse, rose to brown pigment.	Slower growth; more raised. Granular to fluffy surface. Often shows shades of rose to purple pigment. Wine red on reverse.
Potato dextrose agar:	
Granular surface. Rose brown pigment on reverse.	Sparse aerial growth. Deep wine red pigment on reverse.
Cornmeal dextrose agar:	
No pigment.	Wine red pigment on reverse.
Morphology:	
Spirals; microconidia. Nodular organs. Chlamydospores. Macroconidia on wort agar.	Spirals rare. Microconidia in thyrses. Nodules rare. Macroconidia on wort rare. Macroconidia usually on rice, blood agar, ascitic fluid or dextrose mediums. (In a later study it was shown that heart-infusion agar favored the development of the macroconidia of *Trichophyton rubrum*. Of 50 strains studied on this medium only one failed to produce the characteristic macroconidia.) (R.W.B.) (**5a**).

These criteria are useful in typical isolates, but again they are notoriously inconstant so that difficulties arise, particularly when, as is not infrequent, pleomorphic and variant forms develop and obscure the cultural picture. The gross appearance of isolates of the same species may vary from one another, and even the same strain may vary with age and with different media. The diagnostic microscopic structures, such as the spirals and the macroconidia, may be absent, and the culture mount then loses its value as an aid for diagnosis. It is because of this inconsistent behavior that no single property can be regarded as reliable for identification.

As its name implies, *T. rubrum* produces a characteristic deep rose purple to wine red pigment best seen on the under side of the colony, and this property has been used as one of its main differentiating points from *T. mentagrophytes*. Lewis and Hopper (**6**) reported their failure to encounter a single strain that did not acquire a rose purple pigmentation when first isolated. Although the authors were able to maintain the original color of many cultures for three years, they admit that certain strains may lose their pigments after repeated subcultures. This loss of ability to elaborate pigment by certain fungi after the primary isolation is well known and is particularly true for *Trichophyton violaceum*. Another complicating observation that may be a source of confusion was made by one of us (R. W. B.) (**5**) that some strains with the morphology of *T. mentagrophytes* acquired the rose purple pigmentation characteristic of *T. rubrum*.

It is of interest then to study the behavior of pigment production by *T. mentagrophytes* and *T. rubrum* as an aid in the specific differentiation of these two pathogenic fungi.

MATERIALS AND METHODS

Cultures: The strains utilized were culled from the collections of Dr. Rhoda W. Benham and Dr. Norman F. Conant. Of the 50 strains of *T. mentagrophytes*, 27 were isolated in the Vanderbilt Clinic, 8 were from the stock collection of Dr. Conant and 15 were strains obtained by the Hopkins group during their investigation of epidermophytosis at Fort Benning, Georgia in 1946. Of the 40 *T. rubrum* cultures, 26 were Vanderbilt Clinic strains, 13 were

from Dr. Conant and one was a Fort Benning strain. Most of the strains from the Vanderbilt Clinic were relatively recent isolates.

Media: The composition of the different media used was as follows:

Sabouraud dextrose agar (Difco)

Bacto-peptone	10 Gm.
Bacto-dextrose	40 Gm.
Bacto-agar	15 Gm.
Water, to make	1000 cc.

Potato dextrose agar (Difco)

Potatoes, infusion from	200 Gm.
Bacto-dextrose	20 Gm.
Bacto-agar	15 Gm.
Water, to make	1000 cc.

Corn meal agar

Corn meal, infusion from	62.5 Gm.
Agar	19 Gm.
Water, to make	1500 cc.

The chemically pure sugars were added to the amount of 2 per cent.

Blood agar base (Difco)

Beef heart, infusion from	500 Gm.
Bacto-tryptose	10 Gm.
Sodium chloride	5 Gm.
Bacto-agar	15 Gm.
Water, to make	1000 cc.

Two per cent C.P. dextrose was incorporated.

Casein hydrolysate agar

Casein hydrolysate (Smaco)	0.05 Gm.
Magnesium sulfate	0.1 Gm.
Potassium phosphate	1.5 Gm.
Dextrose	50.0 Gm.
Agar	15.0 Gm.
Water, to make	1000 cc.

Ammonium chloride dextrose agar

Ammonium chloride	2.5 Gm.
Magnesium sulfate	0.5 Gm.
Potassium phosphate	1.5 Gm.
Dextrose	20.0 Gm.
Agar	15.0 Gm.

Methods: The different agar media were plated in approximately 10 cc. amounts on small petri dishes of 60 mm. diameter. Inoculations were performed by planting a tiny piece of the fungus colony on the center of the agar plate. The cultures were kept at room temperature and observations made on the first, third and fifth weeks after inoculation. Particular attention was paid to the undersurface pigmentation, the gross appearance and the size of the colonies.

EXPERIMENTAL RESULTS

Pigment Production on Various Media: The 50 strains of *T. mentagrophytes* and 40 of *T. rubrum* were each planted on Sabouraud dextrose agar, potato dextrose agar, corn meal dextrose agar, and ammonium chloride dextrose agar, and the pigment

production noted. Similar observations were made on heart infusion (blood agar base) dextrose and casein hydrolysate dextrose agar using 4 selected strains of each species. Table I summarizes the results of these observations.

TABLE 1

UNDERSURFACE PIGMENTATION OF *T. mentagrophytes* AND *T. rubrum* ON VARIOUS MEDIA

Medium	*T. mentagrophytes*	*T. rubrum*
Sabouraud dextrose agar	Yellow to yellow tan to rose tan	Red brown to red purple to wine red
Potato dextrose agar	Cream to light tan to rose purple	Red purple to intense wine red
Casein hydrolysate dextrose agar	Yellow to tan areas	Red purple to wine red
Blood agar base dextrose	Yellowish tan to deep tan with rose tan to rose purple central area	Yellow to yellow tan with purple to purple brown central area
Corn meal dextrose agar	No pigmentation	Red purple to wine red in 95 per cent of the cultures
Ammonium chloride dextrose agar	Cream to yellow	Cream to yellow

On Sabouraud dextrose agar, the early pigmentation of *T. mentagrophytes* colonies appeared as yellow to yellow-tan to rose-tan which deepened with age approaching the red-purple color of *T. rubrum* in some strains. The earlier pigmentation of many strains of *T. rubrum* colonies was not characteristic, ranging from yellowish cream to dull brown, but it eventually acquired the characteristic purplish hue. The color usually started at the center and gradually spread toward the enlarging edges of the colony. The pigment in some cultures became diffuse throughout. The others left a yellow-tan to rose-tan border.

On potato dextrose agar, the undersurface color of the *T. mentagrophytes* cultures ranged from cream to light tan to rose-tan, and even to actual rose-purple in a few strains. These last five strains were noteworthy for they acquired, particularly in older cultures on potato dextrose agar, a pigmentation that approached and was easily confusable with the red-purple pigmentation of *T. rubrum*. On this medium, the undersurface pigment of *T. rubrum* was most intense with a more pronounced purplish tint.

On corn meal dextrose agar, two strains of *T. rubrum* out of 40 produced only a pinkish undersurface coloration, but the rest acquired a red-purple to intense wine red pigmentation which, however, diffused throughout the undersurface only in a few strains. The difference between the two species on this medium is enhanced by the observation that not one of the 50 strains of *T. mentagrophytes* produced any appreciable undersurface pigment.

Pigment Production on Different Sugars: The media used were the basic corn meal agar with the following C.P. sugars incorporated: dextrose, inulin, raffinose, lactose, sucrose, maltose, mannose, galactose and levulose. Fifteen strains of *T. rubrum* and five of *T. mentagrophytes* were selected and inoculated each on a series of the above corn meal sugar agar media. Satisfactory growths were obtained in all the mediums except on corn meal inulin agar where the growth was relatively slow and limited.

The red-purple pigmentation produced by *T. rubrum* on corn meal dextrose agar appeared similarly on corn meal agar containing mannose and levulose. Twelve strains of *T. rubrum* produced no pigment on the media with inulin, raffinose, lactose, sucrose, maltose and galactose. Three strains acquired a slight pink tinge at the center after five weeks' growth on all the sugar media except corn meal inulin agar. This might be explained by traces of effective sugars in the corn meal used.

No pigmentation at all was seen on any corn meal sugar medium inoculated with the five *T. mentagrophytes* strains.

Pigment Production on Media Buffered at Different pH Levels: Except for the blood agar base which had a pH of about 6.5, the hydrogen ion concentration of the media ranged from pH 5.5 to 6.0. For this particular experiment, one selected strain of *T. mentagrophytes* (*gypseum*) and two of *T. rubrum* were inoculated on a series each of Sabouraud dextrose agar, potato dextrose agar, blood agar base dextrose, casein hydrolysate dextrose agar, corn meal dextrose agar and ammonium chloride dextrose agar buffered with phosphate buffers at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.

With the exception of corn meal dextrose and the casein hydrolysate agar, essentially the same amount and type of growth and pigmentation as obtained on the unbuffered control was observed on

all the other media in the whole series of hydrogen ion concentrations from pH 4.0 to 8.0. The red-purple pigmentation appeared on Sabouraud dextrose and potato dextrose agar buffered at pH 4.0 as well as at pH 8.0. On corn meal dextrose agar, however, there was noticeable retardation of growth at pH 8.0, especially marked in the *T. rubrum* colonies. The purplish hue of *T. rubrum* failed to develop on corn meal dextrose agar at pH 4.0 and pH 4.5 and on casein hydrolysate dextrose agar at pH 4.0 only cream to yellow pigmentation appeared. The colonies on these two media at the rest of the pH levels acquired the undersurface purple pigment.

REMARKS

The media commonly used and found most satisfactory for the growth of the dermatophytes are highly complex with variable and unknown composition of nitrogenous compounds, carbohydrates, minerals and vitamins, each of which, as well as the proportions in which they occur, may affect in varying degrees the ability of the fungi to produce pigments. The importance of dextrose in the pigment production by *T. rubrum* was demonstrated and emphasized, particularly by Lewis and Hopper (**6**). Edgecombe (**7**) was unable to obtain distinct pigment formation with *T. rubrum* on corn meal agar without any added sugar. Likewise, Weidman and Spring (**8**) failed to develop pigments on Grutz medium with peptone as nitrogen source and glycerin, instead of dextrose, as the source of carbohydrate requirements, but succeeded in doing so abundantly, even with ammonium lactate as the only nitrogen source so long as dextrose was present. They stated that the pigment of *T. rubrum* appeared in the presence simply of nitrogen and dextrose.

On the other hand, the 40 strains of *T. rubrum* here reported produced no pink or red pigment in the presence of dextrose when ammonium chloride was the source of nitrogen. This indicates that not only the type of carbohydrate, but also the nitrogen source determines pigment production. Robbins and Ma (**9**) in studying the growth factors for *T. mentagrophytes* observed yellow pigmentation in their basic medium with alanine added, reddish brown with

glycine, valine, histidine, and serine and brown with arginine and asparagine.

Several investigators, including Mosher *et al.* (**10**), Robbins and Ma (**9**) and Archibald and Reiss (**11**), have reported significant differences in growth of pathogenic fungi on mediums containing different amino acids or their combinations. Preliminary experiments of our own with a few strains of *T. mentagrophytes* and *T. rubrum* showed that different amino acids and combinations of them used as nitrogen sources do affect pigment production. It is planned to continue the study of this question.

While the presence of vitamins, especially thiamine, is generally accepted as desirable for growth, these essential growth factors do not seem to influence pigment production decisively. Lewis and Hopper (**6**) reported that the incorporation of vitamins A, B complex, B_1, B_2, G, biotin, C and D in a medium with lactose as the only source of carbohydrate did not permit pigment production by *T. rubrum*.

Another factor to be considered in pigment formation is the mineral content of the substrate. Foster (**12**) discussed the relationships between metal assimilation and pigment production by fungi in his review and Nickerson and Williams (**13**) advised investigations of the dermatophytes along these lines.

The results with the different sugars essentially confirm those of Lewis and Hopper (**6**). Three of the monosaccharides tested served to produce pigment, one (galactose) did not; another evidence of the specificity of enzymes for each optical isomer. In their growth experiments with *T. gypseum*, Goddard (**14**) and Mosher *et. al.* (**10**) failed to demonstrate lactase, and Tate (**15**), invertase, zymase or inulase. None of the di- or polysaccharides were utilized for pigment formation although all but inulin aided growth. Inference may be made that the enzyme systems responsible for the elaboration of pigment may be different from those necessary for growth.

Much study has been made of the influence of hydrogen ion concentration on the growth and pigmentation of dermatophytes. Tate (**16**), Goddard (**14**), Peck and Rosenfeld (**17**) and Leise and James (**18**) showed that the dermatophytes they examined were

not fastidious as to their pH environments and can grow quite easily in a wide range of hydrogen ion concentrations from pH 3.5 to pH 11.0. Lewis and Hopper (**6**) were unable to note any appreciable difference in pigment production by *T. rubrum* when using peptone crude dextrose agar at pH 4, 5, 6, and 7. In the present investigation, changes in hydrogen ion concentration within the range of pH 4.0 to pH 8.0 also failed to affect notably the pigmentation on Sabouraud dextrose agar, potato dextrose agar, and blood agar base dextrose. This, however, was not true of corn meal dextrose agar and casein hydrolysate dextrose agar. As already noted, *T. rubrum* failed to develop its purple pigment in the most acid of these media, although it acquired a cream to yellow color. Nickerson (**19**) observed the development of a yellow color in a mixed culture of *T. rubrum* and *Candida albicans* on corn meal agar when the medium was acid; however, this same strain of *T. rubrum*, when grown alone on corn meal agar, produced the red color when the medium was alkaline. It is noteworthy that the cream to yellow pigmentation observed at the undersurface of the colonies of *T. rubrum* on a medium initially at a pH of 4.0 and 4.5 changed to purple or lavender upon dropping sodium hydroxide solution on the colony. Tate (**16**) extracted the pigments from *T. rubrum* and *T. gypseum* and found them to be yellow in acid solution changing to red or reddish brown in alkaline solution. Thompson (**20**) confirmed Tate's further findings that in alkaline solution the dermatophyte pigments had a yellow, reduced, and a red, oxidized form. Both color changes accompanying differences in pH and in oxidation-reduction potentials are easily reversible. Nickerson (**19**) considered the possibility that the dermatophytes produce only one pigment with the pH and the oxidation-reduction potential of the culture determining the characteristic color. This is rather intriguing in the light of the marked variations of the behavior and appearance of pigment production among dermatophytes in the different media and warrants further investigations.

As regards the practicality of the results of the present investigation in the differentiation of *T. mentagrophytes* and *T. rubrum*, the differences in the undersurface pigmentation of the two species on corn meal dextrose agar may be used as a diagnostic aid. It seems

that the purple pigmentation of *T. rubrum* is developed most intensely and characteristically on potato dextrose agar, and to a lesser degree on Sabouraud dextrose agar, as observed by Lewis and Hopper (**6**) and Edgecombe (**7**); but on the other hand, atypical strains of *T. mentagrophytes* may acquire reddish pigmentation on these media that may cause confusion. Although the purple pigmentation on corn meal dextrose agar was marked, *T. mentagrophytes* acquired no pigmentation at all while *T. rubrum* developed a red, or at least pinkish pigmentation on the undersurface. This should be a help in differentiating atypical strains of *T. mentagrophytes* and *T. rubrum* when other features are indecisive.

SUMMARY

1. The pigment production of 50 strains of *T. mentagrophytes* and 40 strains of *T. rubrum* was studied on Sabouraud dextrose agar, potato dextrose agar, corn meal dextrose agar and ammonium chloride dextrose agar. It appeared that the appearance of pigment on the undersurface depended not only on the presence of dextrose, but also on the source of nitrogen.

2. The strains of *T. mentagrophytes* examined did not produce any pigment on corn meal dextrose agar while those of *T. rubrum* developed the characteristic red purple color in 38 of the 40 strains examined. The other two strains produced pinkish pigmentation on the undersurface not seen on any strain of *T. mentagrophytes*. This observation is suggested as an aid in differentiation of the two species.

3. *T. rubrum* was able to utilize dextrose, mannose and levulose for pigment production, but failed to do so with inulin, raffinose, lactose, maltose, sucrose and galactose.

4. Pigment production by the strains studied on Sabouraud dextrose agar, potato dextrose agar and blood agar base dextrose buffered at different pH levels from pH 4.0 to 8.0 was not appreciably affected although the *T. rubrum* cultures on casein hydrolysate dextrose agar at pH 4.0 and on corn meal dextrose agar at pH 4.0 and 4.5 acquired only a cream to yellow pigmentation instead of red purple which appeared at other pH levels.

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NEW SPECIES OF TORRUBIELLA, HIRSUTELLA AND GIBELLULA[1]

E. B. MAINS

(WITH 2 FIGURES)

Torrubiella pulvinata sp. nov. (FIG. 1, A & B)

Mycelium album, corpus hospitis tegente et ad articulos floccos creante; perithecia partim immersa vel superficialia in pulvino mycelii, ovoidea, 450–600 × 220–270 μ, brunnea, a tenui tunica flavi mycelii tecta; asci cylindrici, 450 × 4.5–6 μ, deorsum attenuata, tenuibus membranis, ad apices membranis spissatis 2.5–3 μ; ascosporae filiformes, 0.5–0.7 μ crassae, multiseptatae, cellis 4–8 μ longis; conidiophora e mycelio, sparsa vel caespitosa, 2–2.5 μ crassa, sursum capitulis phialidum; phialides anguste fusoideo-ovoideae, sursum acuminatae, 8–12 × 2 μ; conidia hyalina, fusoideo-ellipsoidea, 2–3 × 1.5–2 μ, catenulata.

Ex Opilionoideis, Waianuka, Oahu, Hawaii. Dec. 9, 1945, D. P. Rogers (2090).

Mycelium white, covering the body of the host and developing tufts on the legs specially at the joints; perithecia partly embedded or superficial on a pulvinate mass of mycelium, ovoid, 450–600 × 220–270 μ, brown, covered by a thin layer of yellow mycelium; asci cylindric up to 450 μ long, 4.5–6 μ wide, narrowing toward the base, the wall thin, thickened at the apex, 2.5–3 μ; ascospores filiform, 0.5–0.7 μ thick, multiseptate, the cells 4–8 μ long; conidiophores arising from the mycelium, scattered or crowded, 2–2.5 μ wide, phialides grouped in whorls or heads in the upper part of the conidiophores or occasionally from short lateral hyphae, narrowly fusoid-ovoid, acuminate above, 8–12 × 2 μ; conidia hyaline, fusoid-ellipsoid, 2–3 × 1.5–2 μ, catenulate.

On Opilionoidea, south fork of Kaukonahua, 1100 ft., Waianauka, Oahu, Hawaii, Dec. 9, 1945, D. P. Rogers, **2090, type**. (Univ. of Mich. and N. Y. Bot. Gard.)

The hosts of this collection are so severely parasitized that accurate determination is difficult. They appear to be arachnids belonging to the Opilionoidea. The conidial stage resembles *Spi-*

[1] Paper from the Herbarium and the Department of Botany of the University of Michigan.

caria longipes Petch. In the latter species the phialides generally develop from short hyphal branches. Petch (**7**) states that *S. longipes* is the conidial stage of *Torrubiella gonylepticida* (Möller) Petch. *T. gonylepticida* differs from *T. pulvinata* in having the

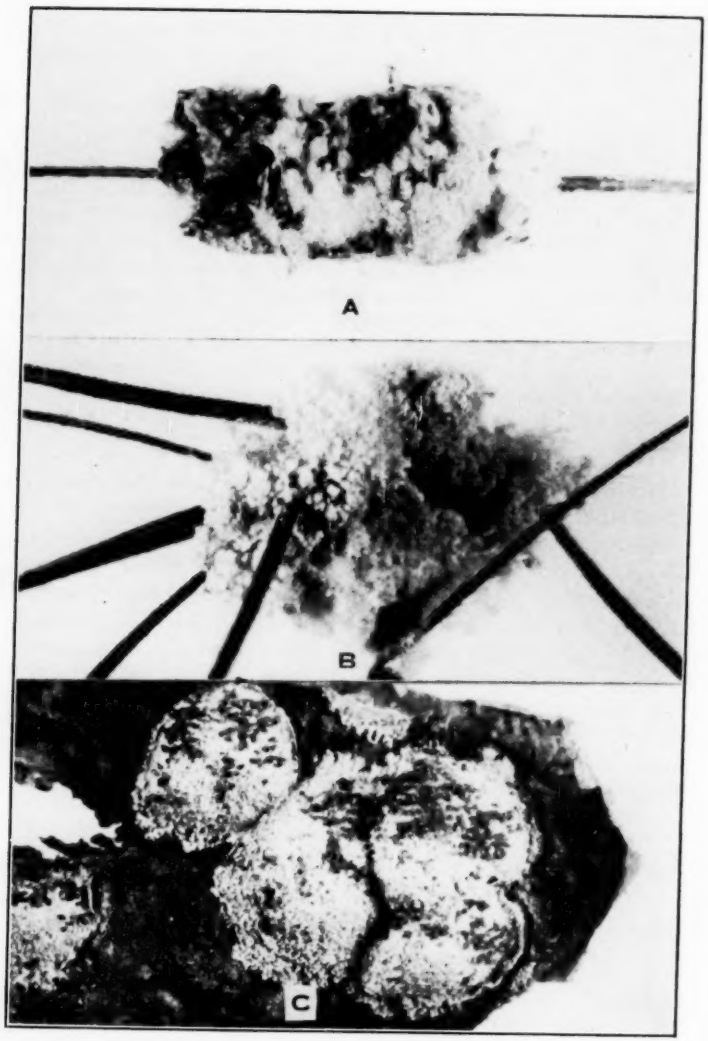

FIG. 1. *Torrubiella pulvinata* and *T. confragosa*.

perithecia scattered over the entire body and appendages of the host developing from a very scanty superficial mycelium. Möller (*2*) describes the perithecia of *T. gonylepticida* as orange red, flask-shaped, 300–400 μ long, and the asci as 170 × 3 μ. Since it is desirable to have a name for the conidial stage, **Spicaria pulvinata** is proposed for that of *T. pulvinata*.

Torrubiella confragosa sp. nov. (FIG. 1, C)

Mycelium tenue, album vel cremeum, hospitem tegente, floccosum, pulverulentum; perithecia irregulariter sparsa vel congesta, superficialia vel paululum immersa, ovoidea, 350–650 × 200–375 μ, rubro-brunnea vel castanea, muris 20–35 μ crassis ex duobus stratis constructis, interno strato rubro-brunneo, externo strato brunneo-flavo; asci cylindric, 200–350 × 3.5–4 μ, tenuibus membranis, ad apices membranis spissatis 2–2.5 μ; ascosporae filiformes, multiseptatae; synnemata summe brevia, 300–800 μ longa, 80 μ crassa, cylindricae, interdum furcata; phialides 14–20 μ longae, deorsum 1.5 μ latae, sursum attenuatae, acuminatis apicibus; conidia oblonga, 3–4 × 1.5–1.7 μ, muco tecta, 2–5 in glebulas congregata.

In Coccidis, Novo Petropolis, Brazil, May 1923, Rick, specimen typicum; Bayeaux, Haiti, J. R. Weir.

Mycelium thin, white to cream color, covering scale insects and extending slightly beyond on the substratum, slightly tufted, pulverulent; perithecia irregularly scattered to crowded over the scale, superficial or slightly embedded at the base in the mycelium, ovoid, 350–650 × 200–375 μ, reddish brown to dark chestnut brown, the wall 20–35 μ thick and consisting of two layers, the inner reddish brown, the outer brownish yellow; asci cylindric, 200–350 × 3.5–4 μ, the wall thin, thickened at the apex, 2–2.5 μ; ascospores filiform, 0.5 μ wide, almost as long as asci, multiseptate, part-spores not seen; synnemata very short, 300–800 μ long, 80 μ thick, cylindric or furcate with short irregular branches, consisting of loosely interwoven hyphae; phialides arising from the outer hyphae or from short lateral hyphae, 14–20 μ long, 1.5 μ wide below, gradually narrowing to an acuminate apex; conidia oblong, 3–4 × 1.5–1.7 μ, produced in a mucus, often in clumps of 2 to 5.

On large scale-insects. Novo Petropolis, Rio Grande do Sud, Brazil, May 1923, Rick, **type** (Univ. Mich. and Farlow Herb.); Bayeaux, Haiti, J. R. Weir (Univ. Mich. and Mycol. Coll. Bur. Plant Ind. Washington, D.C.).

Both of these collections were received as *Torrubiella rubra*. *T. rubra* develops perithecia only at the margin of the mycelium cover-

ing the scale or on a hypothallus and the asci are much larger than those of *T. confragosa*. Petch (**3**) describes a conidial stage for *T. rubra* which differs considerably from that of *T. confragosa*. The latter can best be classified as a *Hirsutella*, although the synnemata are poorly developed. The scattered phialides and clumped spores in a surrounding mucus are characteristic of that genus. Since the conidial stage of fungi of this group frequently develop without the perithecial stage it is convenient to have a name applying to it and consequently **Hirsutella confragosa** is proposed.

Torrubiella liberiana sp. nov. (FIG. 2, A)

Perithecia albido-brunnea, ex myceliis in articulis hospitis erumpentia, partim immersa, ovoidea, 300–400 × 150–200 μ; asci cylindrici, tenues, 210 μ longa, 3–4 μ crassa, tenuibus membranis, ad apices membranis spissatis, 2 μ; ascosporae filiformes, multiseptatae, fragentes, segmentis 3–4 × 0.5–0.7 μ.

Ex formica, Bonata, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr.

Perithecia light brown, developing in small white patches of mycelium at the joints of the appendages and of the body, partly embedded, ovoid, 300–400 × 150–200 μ; asci cylindric, slender, up to 210 μ long, 3–4 μ wide, wall thin, thickened at the apex, 2 μ; ascospores filiform, nearly as long as the asci, multiseptate, breaking into one-celled fragments, 3–4 × 0.5–0.7 μ.

On an ant. Bonata, Central Province, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr. (Univ. Mich.), **type**.

As far as the writer is aware, this is the first report of a species of *Torrubiella* on ants. It occurs in close association with synnemata of a *Hirsutella* which may be the conidial stage. However, it was separated from a collection of ants which were mostly parasitized by *Cordyceps australis*. A *Gibellula* also occurs on a few specimens. The relationship of these fungi is therefore very uncertain and it seems best to describe them separately.

Hirsutella liberiana sp. nov. (FIG. 2, B)

Synnemata pauca, sparsa, ex exiguis albidis myceliis plerumque in articulis erumpentia, albida, tenuia, cylindrica, 3 mm. longa, deorsum 100 μ crassa, sursum paululum attenuata, simplicia vel interdum furcata; phialides sparsae vel congregatae, plerumque nonnullae ex brevibus lateralibus hyphis erumpentes, deorsum fusoideo-ellipsoideae vel fusoideo-oblongae, 8–12 × 3 μ,

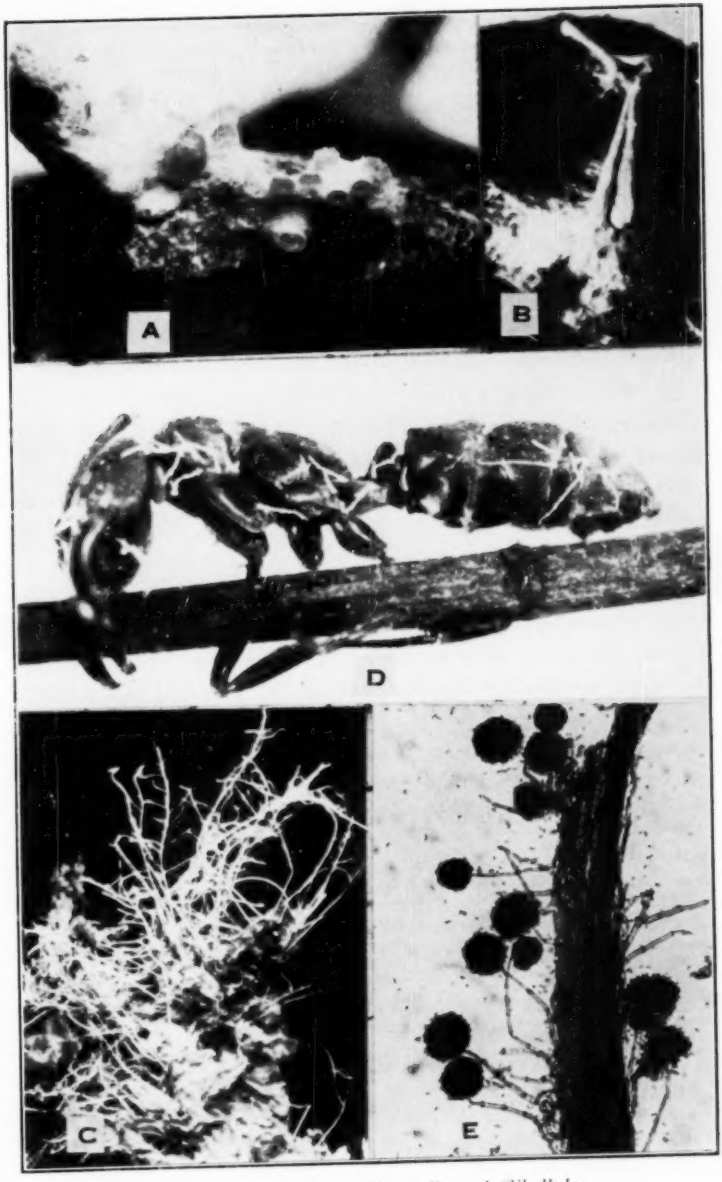

FIG. 2. *Torrubiella*, *Hirsutella* and *Gibellula*

sursum attenuatae quisque 10–14 μ longo sterigmate; conidia cylindrica, 3–4 × 0.7–1.0 μ; muco tecta, in glebulas congregata.

Ex formica, Bonata, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr.

Synnemata few, scattered, arising from a slight whitish mycelium, usually from the joints of the body and the appendages, whitish, slender, cylindric, slightly narrowing upward, up to 3 mm. long, 100 μ thick, simple or occasionally branched above; phialides scattered to crowded, usually several arising from short, one-celled lateral hyphal branches from the outer hyphae of the synnemata, the lower part fusoid-ellipsoid to fusoid-oblong, 8–12 × 3 μ, the upper part prolonged into an attenuated, acuminate sterigma, 10–14 μ long, each producing several conidia which adhere together in a persistent mucus; conidia cylindric, rounded at both ends, 3–4 × 0.7–1.0 μ.

On an ant, Bonata, Central Province, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr. (Univ. Mich.), **type**.

Petch (**6**) has described *Hirsutella formicarum* on ants from British Guiana and Ceylon and has stated that it is the conidial stage of *Cordyceps unilateralis*. He describes the conidia as narrowly cymbiform, 9–11 × 2 μ. In an earlier publication (**4**) he described the conidia of the Ceylon specimens as oval, 3–5 × 1 μ. Kobayasi (**1**) has identified Japanese collections on ants as *H. formicarum*, describing the conidia as ovoid, ellipsoid or fusiform, 3–4.2 × 1.5–2 μ. It would seem probable that more than one species is concerned. *Hirsutella liberiana* is distinguished by its cylindric narrow conidia. It was associated with perithecia of *Torrubiella liberiana* which is described herein.

Hirsutella ramosa sp. nov. (FIG. 2, C)

Synnemata multa, gregaria, tenuia, irregulariter ramosa, 8 mm. longa, deorsum 52–140 μ crassa, sursum attenuata, accuminatis apicibus, deorsum albido-flava, sursum alba, multis albis rectis vel obliquis ramulis; phialides sparsae vel 1–3 caespitosae, hyalinae, subulatae, 19.3–36.4 μ longae, deorsum 2.1–3.2 μ crassae, sursum attenuatae, acuminatis apicibus; conidia oblonga, 3.2–5.5 × 1.1–1.4 μ, tenui muco tecta, in parvas globosas glebulas 4.3–6.4 μ dia. congregata.

Ex larva lepidopteri, Salmon River, Nova Scotia, Sept. 7, 1931, L. E. Wehmeyer (1474).

Synnemata numerous, arising from all parts of the host, slender, irregularly branched, the main stem up to 8 mm. long, 52–140 μ

thick at the base, gradually narrowing to acuminate apices, light yellow below, white above, the branches numerous, white, at right angles or slightly oblique to the main stem, occasionally producing secondary branches, the hyphae in the stem and branches longitudinal and parallel, multiseptate, 2 μ wide; phialides hyaline, minutely roughened, subulate, 19.3–36.4 μ long, 2.1–3.2 μ wide at the base, gradually narrowing to an acuminate apex, scattered on the upper part of the stem and branches giving a setose appearance, arising singly directly from a cell of the outer hypha of the synnema or 1–3 at the apex of short, 6.4–10.7 μ, laterally projecting cylindric hyphae; conidia oblong, 3.2–5.5 × 1.1–1.4 μ, covered with a slight mucus, adhering to form small, 4.3–6.4 μ, spherical clumps.

On fragments of a lepidopterous larva on a mossy log, Salmon River, Nova Scotia, Sept. 7, 1931, L. E. Wehmeyer, **1474, type** (Univ. Mich.).

The much branched synnemata and narrow subulate phialides distinguish *H. ramosa* from other species of *Hirsutella* infecting Lepidoptera.

Gibellula formicarum sp. nov. (FIG. 2, D & E)

Synnemata sparsa, plerumque ex articulis hospitis erumpentia, albido-brunnea, anguste cylindrica, 1–2 mm. longa, 50–150 μ crassa, exterioribus hyphis laxis brunneis asperulatis; conidiophora 50–150 μ longa, 2-4-septata, inferioribus cellulis 3–5 μ latis, asperulatis, superiore cella obovoidea, 7–8 × 4–8 μ, levi, hyalina, globosum vel cylindricum capitulum prophialidium et phialidium gerentis; prophialides ellipsoideae vel subglobosae, 3.5–4 × 2.5–3 μ, quisque paucam phialidem gerentis; phialides ovoideae vel cylindricae, 4–14 × 1.5–2.5 μ; conidia hyalina, cylindrica, 3–4.5 × 1–1.5 μ.

Ex formicis, Belleyella-Kondessu-Zui, Liberia, Dec. 1947, J. T. Baldwin, Jr., specimen typicum; Bonata, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr.

Synnemata scattered, arising from various parts of the body and appendages of the host, usually at the joints, very light brown, narrowly cylindric, 1–2 mm. long, 50–150 μ thick, composed of longitudinal somewhat interwoven hyphae, the outer, loose, brown, asperulate; conidiophores arising from short lateral prolongations of cells of the outer hyphae, 50–150 μ long, 2–4-septate, the lower cells 3–5 μ wide, the walls brownish, asperulate, the terminal cell obovoid, 7–8 × 4–8 μ, smooth, hyaline, bearing a globose to cylindric head of prophialides and phialides; prophialides ellipsoid to subspherical, 3.5–4 × 2.5–3 μ, each bearing several phialides; phialides ovoid to cylindric, 4–14 × 1.5–2.5 μ; conidia hyaline, cylindric, rounded at the ends, 3–4.5 × 1–1.5 μ.

On ants, along the route Belleyella-Kondessu-Zui, Western Province, Liberia, Dec. 8, 1947, J. T. Baldwin, Jr. (Univ. Mich.).

The specimens were included in collections of *Cordyceps australis* (Speg.) Sacc. which were received from Dr. Baldwin. The collections were composites collected from several localities. The synnemata of *Gibellula formicarum* were not found associated with clavae of *C. australis* upon the same insect and therefore the ascogenous stage is uncertain. Petch (**5**) has reported that the conidial stage of *C. australis* is an *Hymenostilbe*.

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EXPLANATION OF FIGURES

FIG. 1. A & B. *Torrubiella pulvinata*. A. Pulvinate mass of mycelium bearing perithecia arising from a leg of the host. × 12. B. Body of a host covered by a mass of mycelium and conidiophores. × 7. C. *Torrubiella confragosa*. Large scale-insects covered with a thin layer of mycelium-bearing synnemata and perithecia. × 4.

FIG. 2. A. *Torrubiella liberiana* showing perithecia developing on a small mass of mycelia at the base of an antenna of an ant. × 25. B. *Hirsutella liberiana* showing synnemata arising from a small patch of mycelium on an ant. × 7. C. *Hirsutella ramosa* showing the branched synnemata. × 7. D & E. *Gibellula formicarum*. D. An ant showing the scattered synnemata on various parts of the body. × 7. E. A portion of a synnema showing capitate conidiophores (stained with nigrosin). × 200.

PHASE-DETERMINING FACTORS IN BLASTOMYCES DERMATITIDIS

S. B. Salvin

(WITH 2 FIGURES)

Blastomyces dermatitidis, the causative organism of North American blastomycosis, is characterized by the development (a) of aerial hyphae with numerous microconidia at room temperature, and (b) of large thick-walled predominantly single-budding cells at 37° C. The mycelial phase has been grown on a wide variety of complex media at room temperature, but the budding or yeastlike phase has been grown principally on beef-infusion or blood agars at 37° C.

The process of conversion from one growth phase to the other has been studied since the turn of this century, but is still not completely understood. Ricketts (**8**) and Hamburger (**4**) were among the first to examine the growth of the fungus "in vitro," and indicated that temperature was a most influential factor in determining the morphologic type. This fact has since been confirmed by others, such as Michelson (**7**), DeMonbreun (**3**), and Levine and Ordal (**6**). Levine and Ordal (**6**) also reported pH as being relatively unimportant in determining the type of growth.

Almost all this work, however, has been done in complex and not chemically defined media. Hence, little is known about the possible influence of nutrient factors on the conversion of the organism from one phase to the other. Investigations were therefore initiated on the nutritional requirements of the yeastlike and mycelial phases. This paper reports the effect of growth factors, amino acids and carbohydrates on the development of *B. dermatitidis* in the mycelial and yeastlike phases.

MATERIALS AND METHODS

The three strains of *B. dermatitidis* used in the present studies were from the collection maintained in this laboratory, and were

originally isolated from cases of human blastomycosis in southeastern United States. The mycelial phases of the fungi were maintained on Sabouraud's dextrose agar at 25° C., while the budding forms were kept on 20 per cent rabbit blood agar at 37° C.

The basic medium used for the study of the nutritional requirements of the strains under investigation was as follows:

Na_2HPO_4 (anhydrous)	5.0 g.
NaCl	4.0 g.
KCl	1.0 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
$CaCl_2$	0.025 g.
$FeCl_3$	0.01 g.
agar	1.5 g.
biotin	50 gamma
folic acid	50 gamma
inositol	10 mg.
niacin	1 mg.
calcium pantothenate	1 mg.
p-aminobenzoic acid	10 mg.
pyridoxine	1 mg.
riboflavin	1 mg.
thiamin	1 mg.
distilled water to make	1000 ml.

When the budding phases were to be grown, 1.8 g./liter of a nitrogen-containing compound and 2.5 g./liter of a sugar were added to the above medium. When the mycelia were to be grown, 14 g./liter of a nitrogen-containing compound and 10 g./liter of a sugar were added. All the studies were made in a semifluid medium, since such a medium promoted better development of the yeastlike phase than an agar slant, and also permitted quantitative determination of the growth. In the experiments in which the growth-factor requirements of the fungi were investigated, the agar was washed for a minimum of 5 days in running tap water, then rinsed in distilled water and dried. The medium, after adjustment to pH 7.0, was generally dispensed in 8 ml. quantities in test tubes, and after inoculation with the fungus incubated either at 37° C. in sealed tubes for 2–3 weeks for the budding phases, or at 25° C. for 6–8 weeks for the mycelial phases. The inoculum was either (a) a loopful of a dense washed suspension of microconidia and mycelial fragments in saline, or (b) 0.05 ml. of a washed suspension of about 1,000,000 cells per ml. When necessary, cells were counted in a Levy haemacytometer.

EXPERIMENTS AND RESULTS

When the yeastlike phase of three strains of *B. dermatitidis* was inoculated into 19 tubes of basic medium each one of which also contained 18 amino acids and from each one of which a different natural amino acid was omitted, incubation at 37° C. for two weeks produced good growth of the budding cells. The amino acids studied were glycine, alanine, serine, threonine, valine, leucine, isoleucine, cysteine, methionine, proline, hydroxyproline, tyrosine, tryptophane, phenylalanine, aspartic acid, glutamic acid, lysine, arginine, and histidine.

TABLE 1

GROWTH OF THE YEASTLIKE PHASE OF *Blastomyces dermatitidis* AFTER TWO WEEKS' INCUBATION AT 37° C. IN A SEMIFLUID MEDIUM CONTAINING A SINGLE NITROGEN-BEARING COMPOUND. (Inoculum: 50,000 yeastlike cells/cc.)

Compound*	Number of cells per cc.		
	Strain 6046	Strain 6021	Strain 6014
ammonium sulfate	75,000	100,000	200,000
glycine	2,100,000	2,250,000	1,275,000
alanine	1,350,000	500,000	500,000
serine	2,350,000	2,125,000	2,150,000
valine	1,000,000	750,000	2,500,000
tyrosine	75,000	100,000	225,000
proline	1,700,000	3,250,000	2,000,000
hydroxyproline	2,750,000	3,500,000	1,875,000
aspartic acid	1,750,000	2,000,000	1,625,000
glutamic acid	1,850,000	2,500,000	3,250,000
arginine	75,000	125,000	200,000

* The media containing threonine, leucine, isoleucine, phenylalanine, cysteine, cystine, methionine, tryptophane, histidine, lysine, or potassium nitrate contained cells in quantities no greater than that in the original inoculum.

In the presence of a single nitrogen-bearing compound (such as one of the amino acids, inorganic ammonium sulfate or potassium nitrate) in the absence of carbohydrate, after inoculation with 50,000 cells of the yeastlike phase and subsequent incubation at 37° C. for two weeks, the amount of growth varied in the different tubes depending upon the nitrogen source (table 1). Extensive development of the yeastlike phase of each of the three strains tested occurred only in the media with glycine, alanine, serine, valine, aspartic acid, glutamic acid, proline and hydroxyproline. Growth

was slight in the tubes in which inorganic nitrogen was the sole source of nitrogen.

In all those cases where the growth was slight, only the yeastlike phase developed. No mycelium or abortive mycelium of any of the three strains studied appeared to any extent during the first

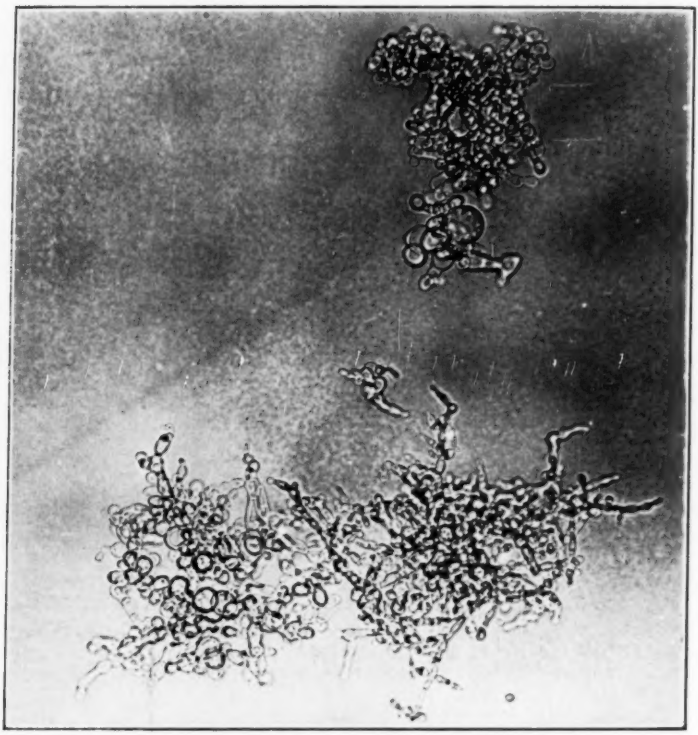

FIG. 1. Abortive mycelium of strain 6014 of *B. dermatitidis* from a culture grown in a peptone semifluid medium for 2 weeks at 37° C.

three weeks of growth at 37° C. in the semifluid medium with pure amino acids. However, abortive mycelia frequently occurred at 37° C. either in a peptone semifluid medium or on 10–20 per cent rabbit blood agar (FIGS. 1, 2).

Tubes containing a single amino acid as the nitrogen source were inoculated with the yeastlike phase of three strains and incubated

TABLE 2

GROWTH OF THE YEASTLIKE PHASE OF *B. dermatitidis* AFTER 2 WEEKS' INCUBATION AT 37° C. IN A SEMIFLUID MEDIUM CONTAINING A SINGLE NITROGEN-BEARING COMPOUND. (Inoculum: about 50,000 conidia and hyphal fragments per cc.)

Compound*	Number of cells per cc.		
	Strain 6046	Strain 6021	Strain 6014
alanine	25,000	300,000	125,000
serine	2,700,000	3,250,000	2,125,000
proline	50,000	225,000	200,000
hydroxyproline	1,300,000	675,000	925,000

* The media containing the other nitrogen compounds contained less than 25,000 cells per cc.

at 25° C. Growth was found to occur in varying degrees on all the amino acids, and was always mycelial in character.

When a suspension of conidia and hyphal fragments was used as the inoculum in the media containing but a single amino acid and the culture incubated at 37° C., the yeastlike phase alone developed where growth did appear (table 2). However, extensive growth of the three strains tested occurred only in the media with either serine or hydroxyproline as the sole amino acids, as well as in the tubes containing a mixture of twenty different amino acids. Moderate development appeared in the media with proline or alanine. In those media where growth was slight, yeastlike cells and no mycelia were found.

Media were prepared in which glutamic acid was the sole source of nitrogen and to which one of nine different growth factors (biotin, folic acid, inositol, nicotinic acid, p-aminobenzoic acid, cal-

TABLE 3

GROWTH OF THE YEASTLIKE PHASE OF *B. dermatitidis* IN A SEMIFLUID MEDIUM CONTAINING GLUTAMIC ACID AS THE NITROGEN-BEARING COMPOUND AND WITH VARYING AMOUNTS OF DEXTROSE, AFTER 1 WEEK AT 37° C. (Inoculum: 50,000 yeastlike cells/cc.)

Amount of dextrose (mg./100 ml.)	Number of cells per cc.	
	Strain 6014	Strain 6046
0	250,000	575,000
10	900,000	950,000
100	2,250,000	1,500,000
200	2,850,000	2,300,000
500	5,200,000	2,650,000
1000	4,250,000	2,000,000

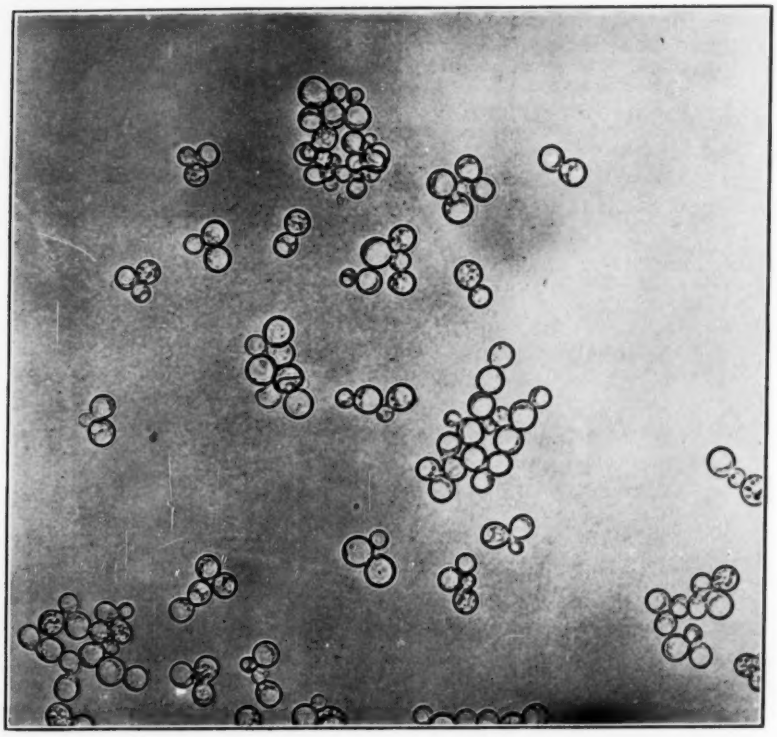

FIG. 2. Budding cells of strain 6014 of *B. dermatitidis* from a 2 weeks' culture (at 37° C.) grown in a semifluid medium with serine as the nitrogen source.

cium pantothenate, pyridoxine, riboflavin and thiamin) was added. Examination after two weeks' incubation at 37° C. revealed about the same number of yeastlike cells in the different media, with no growth factor having any discernible stimulatory effect on the three strains tested.

The yeastlike phase was inoculated into media with glutamic acid as the nitrogen source and with the carbohydrate dextrose varying from 0 to 1000 mg per liter. Budding cells of the yeastlike phase again were the only ones to appear at 37° C., with the optimum concentration of dextrose being 500 mg. (table 3).

When this amount of carbohydrate (5 g./l.) was used in similar

media, and one of 28 different sugars substituted for the dextrose, there was a marked difference in the extent of growth, although some did appear in all the sugars. However, what growth did appear was yeastlike.

DISCUSSION

The phenomenon of dimorphism, whereby a single species can appear in two distinct morphologic phases, is quite common among the pathogenic fungi. For example, *Histoplasma capsulatum* appears in the yeastlike phase at 37° C., and in the mycelial at 25° C. *Blastomyces brasiliensis* develops as a multiple budding cell at 37° C., and as a mycelium at 25° C. *Coccidioides immitis* occurs as an endospore-forming spherule in the tissue, and as mycelium at 25° C.[1] *Candida albicans* has been grown in both the yeastlike and mycelial phases at both 25° C. and 37° C., with the most influential morphology-determining factor or factors still in doubt. *Phialophora verrucosa* appears as round, dark brown bodies in the tissue, and as mycelium in culture.

In some species, such as in *Phialophora verrucosa* and *Coccidioides immitis*, the mechanism is not understood. In others, such as *Candida albicans* and *Histoplasma capsulatum*, some experimentation has provided a partial explanation of the processes involved. The yeastlike phase of *H. capsulatum* does not always occur at 37° C., but must have certain other factors present (**9**). (a) Biotin must be an ingredient of the medium. (b) A reduced-sulfur group such as the sulfhydryl group must be included. Thus, conversion of the yeastlike phase to the mycelium, and vice versa, may be brought about at 37° C. by changing the amino-acid constitution of the medium.

Evidence has been accumulating that temperature is the principal controlling factor in *B. dermatitidis* (**2, 3, 6**). However, all this work, with the exception of some brief studies by Levine and Ordal (**6**) in which ammonium sulfate was the nitrogen source, has been done in complex media the exact chemical composition of which was not known. Such media included primarily various beef

[1] The tissue or spherule phase has not been grown extensively "in vitro" (**1, 5**).

infusion, peptone or blood agars, and did not permit proper evaluation of the possible effect of the nutrients present in the medium.

In simple chemically-defined media, however, temperature proved still to be the main factor determining growth of mycelia and conidia, or growth of large budding cells. At 25° C., growth was always mycelial; at 37° C., growth was yeastlike, or occasionally consisted of abortive mycelium (FIG. 1). No growth factor was found capable of either (a) stimulating growth of either phase or (b) stimulating conversion of one phase to the other. Although the yeastlike phase developed slightly in the medium containing the ammonium ion as the sole nitrogen source, optimum growth appeared in a medium with amino acids. Glycine, alanine, serine, valine, glutamic acid, aspartic acid, proline and hydroxyproline stimulated development most.

These phase-determining characteristics of *B. dermatitidis* are much like those of *B. brasiliensis* (**10**). (a) The yeastlike phase of *B. brasiliensis* appeared at 37° C. in the amino-acid media, with glycine, serine, glutamic acid, aspartic acid, proline and hydroxyproline producing most growth. However, growth was much slower, more limited in extent, and reverted more readily to an abortive-mycelium type. (b) Serine and hydroxyproline proved the most stimulatory of the amino acids, especially in converting conidia to budding cells of the yeastlike phase at 37° C. (c) No growth factor was found to have any effect at 37° C. (d) Growth occurred with or without carbohydrate in the medium. This similarity in growth characteristics between *B. dermatitidis* and *B. brasiliensis* parallels their morphologic similarities, and adds further evidence to the beliefs of Conant and Howell (**2**) that the two organisms belong in the same genus and are very close phylogenetically.

It now seems certain that the morphology of *B. dermatitidis*, as well as of *B. brasiliensis*, is controlled primarily by temperature changes. *H. capsulatum*, however, is determined not only by temperature but also by nutrient factors in the medium. Studies are being conducted to determine what factors are most effective in regulating the phases of other dimorphic pathogenic fungi.

SUMMARY

The yeastlike phase of *B. dermatitidis* was grown in a chemically-defined semifluid medium. Temperature was the most important environmental factor controlling the type of growth. No amino acid, carbohydrate or "growth substance" tested was found to be an essential accessory factor for growth of the yeastlike phase at 37° C. However, the extent of growth of the yeastlike phase did vary greatly according to the constitution of the medium.

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THE PERFECT STAGE OF THE FUNGUS CAUSING SPOT ANTHRACNOSE OF ARBUTUS UNEDO L.

G. Arnaud and A. A. Bitancourt

(with 1 figure)

As shown by Jenkins [1] the causal fungus of spot anthracnose of the strawberry-tree *Arbutus Unedo* L. was first described by P. A. Saccardo and D. Saccardo [2] under the name of *Illosporium Mattirolianum* and included in D. Saccardo's "Mycotheca italica" under number 798. Briosi and Cavara included in their exsiccati "I funghi parassiti delle piante coltivate od utili" under number 349 a specimen of the same organism and identified it as *Hadrotrichum Populi* Sacc. forma *Arbuti*. Jenkins (l.c.) studied and illustrated both specimens and showed that the fungus belonged in the genus *Sphaceloma* and accordingly made the new combination *S. Mattirolianum* (Sacc. & D. Sacc.).

Some time ago, in a routine examination of Briosi and Cavara's specimens in their set of fungi exsiccati (l.c.) in the "Centre de Recherches Agronomiques," Versailles, France, the senior author found on the lesions of number 349 several ascomata of *Elsinoë* in the vicinity of the acervuli and sporodochia of the *Sphaceloma*.

The writers have now completed a more detailed study of this ascomycete which has enabled them to prepare the following description:

Ascomata are seen on the surface of the leaf spot as small, buff colored prominences; in transverse section they are intraepidermal, erumpent, covered with the remnants of the outer wall of the leaf epidermis, and made up of a dark pseudoparenchyma, darker in the upper layers, which form a more or less well-defined epithecium, lighter colored in the deeper layers, which are almost hyaline at the

[1] Jenkins, A. E. 1933. Additional studies of species of *Elsinoë* and *Sphaceloma*. Mycologia **25**: 213–220.

[2] Saccardo, P. A. 1902. Sylloge Fungorum **16**: 1093.

base and are seated directly on the upper part of the cells forming the palisade parenchyma of the host. They are 80–50 μ in diameter and 40–60 μ thick. The asci are distributed in one layer in the upper part of the ascoma and are ovoid or club-shaped, averaging $30 \times 18\ \mu$. The ascospores are hyaline, 1-septate—probably on account of being immature—with the upper cell shorter and broader than the lower cell, constricted at the septum, and usually measure $11 \times 4\ \mu$.

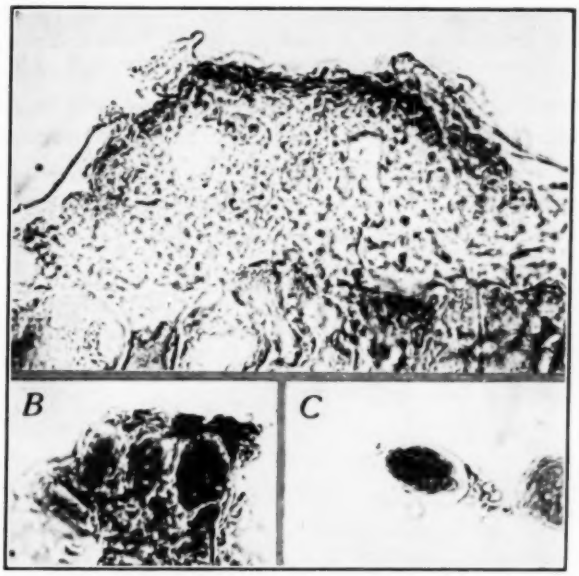

FIG. 1. *Elsinoë Mattirolianum*. A, ascoma; B, three asci in place in an ascoma; C, ascus with ascospores. × 500. Photo. A. A. Bitancourt.

In all the fungi of which the imperfect stage belongs in the genus *Sphaceloma* and the perfect stage is known, the latter pertains to the genus *Elsinoë*. The presence on the same lesions of acervuli and sporodochia of *Sphaceloma* and of ascomata of *Elsinoë* is at least circumstantial evidence that both are part of the life cycle of the same organism. It appears therefore legitimate to consider the ascomata found in the lesions of the spot anthracnose of *Arbutus Unedo* as those of *Sphaceloma Mattirolianum*.

Elsinoë Mattirolianum sp. nov.

Ascomatibus intraepidermicalibus erumpentibus, 80–150 × 40–60 μ pseudoparenchymatosis e cellulis superne nigricantibus, epithecium formantibus, interne pallidioribus constitutis. Ascis ellipticis octosporis, 30 × 18 μ; ascosporis hyalinis, uniseptatis (immaturis?) medie constrictis, 11 × 4 μ.[3]

Status conidiiferus:

Sphaceloma mattirolianum (Sacc. & D. Sacc.) Jenkins (Mycologia **25**: 214. 1933).

Syn. *Illosporium mattirolianum* Sacc. & D. Sacc. (Sacc. Syll. Fung. **16**: 1093. 1902).

Hadrotrichum populi Sacc. forma *Arbuti* Briosi & Cav. (Fung. parass. piante colt. od ut., number 349), cum ic. 1900.

Material of the perfect stage examined: On leaves of *Arbutus Unedo* L. associated with the imperfect stage as collected at Bari, Italy, by Montagna and distributed by Briosi and Cavara (Fung. parass. piante colt. od ut., number 349) under the name *Hadrotrichum Populi* forma *Arbuti*.

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[3] Translated in Latin by D. B. Pickel.

NOMENCLATORIAL PROBLEMS IN THE PERONOSPORACEAE [1]

CHARLES GARDNER SHAW [2]

(WITH 2 FIGURES)

Whether Article 57 of the International Rules of Botanical Nomenclature (**2, 3**), as now worded, applies to the Phycomycetes or not has been a subject of considerable controversy (**1, 6**). The first paragraph of Article 57, which consists of two sentences, is the primary cause of this controversy.

The English version of the first sentence reads as follows: "Among Fungi with a pleomorphic life-cycle the different successive states of the same species (*anamorphoses, status*) can bear only one generic and specific name (binary), that is the earliest which has been given, starting from Fries, *Systema*, or Persoon, *Synopsis*, to the state containing the form which it has been agreed to call the perfect form, provided that the name is otherwise in conformity with the Rules." I consider this sentence to contain the intent of the article and to be unequivocal.

The second sentence of the first paragraph merely defines the perfect state of certain groups of the fungi. It reads as follows: "The perfect state is that which ends in the ascus stage in the *Ascomycetes*, in the basidium in the *Basidiomycetes*, in the teleutospore or its equivalent in the *Uredinales*, and in the spore in the *Ustilaginales*." That the Phycomycetes are not mentioned in this second sentence, in the absence of evidence to the contrary, must be considered an unintentional omission, which in no way influences the meaning of the first sentence. No other interpretation of the article, as now worded, seems possible to me.

[1] Based on a portion of a thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Assistant Professor and Assistant Pathologist, Division of Plant Pathology, Washington State College. Published with the approval of the Director of the Washington Agricultural Experiment Station as Technical Paper No. 805.

In the Phycomycetes, and particularly in the Peronosporaceae, the "perfect state" has been used sparingly in classification. With few exceptions identifications can be made, and normally are made, on the imperfect or asexual stages alone. Indeed, in the absence of conidiophores and conidia, species of the Peronosporaceae have been placed in the genus *Protomyces* [*e.g. Protomyces graminicola* Sacc. = *Sclerospora graminicola* (Sacc.) Schroeter; *Protomyces fuscus* Peck = *Plasmopara pygmaea* (Unger) Schroeter].

Numerous mycologists, perhaps the majority, would prefer not to apply Article 57 to the Phycomycetes. Bisby (**1**) has proposed a rewording of this article which would exclude the Phycomycetes. Bisby (**2**, p. 89) enounces that a proposal, once made, can be followed "unless and until Congress decides against a proposal now before it." I would interpret the Rules differently.

It seems to me that Bisby's proposal and all others are simply recommendations which have no standing according to the Rules until acted upon by an International Botanical Congress. If accepted, they still remain on trial until sanctioned, emended, or rejected by the second successive Congress (Art. 74). Since there is no assurance that any given proposal will be accepted or even acted upon by the next Congress, and since there is no absolute certainty as to when the Congress will again convene, the interests of nomenclatorial stability (Art. 4) are not furthered by following proposals before they are considered by the Congress.

Before any change in the Rules is adopted, consideration should be given to the effect the proposed change would have on the nomenclature of the groups concerned (**8**). The purpose of this paper is to point out (1) the proper binomials and author citations for certain species of the Peronosporaceae according to the present wording of Article 57 (these binomials are used as headings for the species discussed); (2) the binomials and author citations in common use for these fungi; (3) the binomials that would be valid if Bisby's proposed change in Article 57 were incorporated into the Rules.

The lists of synonyms that follow are not intended to be complete. Only those pertinent in answering the problems indicated above are included. Each synonym is listed exactly as given in the original paper cited. Familiarity with the Botanical Rules of

Nomenclature should make apparent the proper binomials and author citations, either according to the present wording of Article 57 or according to Bisby's proposal. Discussion has therefore been limited to those cases of special interest.

Finally it must be pointed out that, in a discussion of nomenclatorial problems, the distinction between taxonomy and nomenclature is important (8). All decisions about status, whether they concern individuals or major groups, fall within taxonomy. Nomenclature relates only to the choice of names to be used after these taxonomic decisions have been made. The two are diametrically opposed—nomenclature aims at stability; taxonomy, from its very nature, is everchanging.

The present paper deals with nomenclatorial problems. A discussion of taxonomic problems, especially the species concept within the Peronosporaceae, is reserved for a later paper.

1. *Basidiophora Kellermanii* (Swingle ex Saccardo) Wilson—Usually cited as *B. Kellermanii* (Ellis & Halsted) Wilson.

Peronospora Kellermanni Ellis & Halsted (pro tem.) in Ellis & Everh., N. Amer. Fungi No. 2201. 1889. (Nomen nudum.)
Plasmopara sp. Swingle in Trans. Kans. Acad. Sci. **11**: 74. 1889. (Oospores.)
Plasmopara Kellermani (Ell. et Halst.) Swingle ex Saccardo, Syll. Fung. **9**: 342. 1891. (Oospores.)
Basidiophora Kellermanii (Ellis & Halsted) Wilson in Bul. Torrey Bot. Club **34**: 394. 1907. (Oospores.)

I believe *Basidiophora Kellermanii* (Swingle ex Sacc.) Wilson most nearly conforms with the Botanical Rules. Since *Peronospora Kellermanni* Ellis and Halsted is a *nomen nudum*, it has no standing. Although Swingle failed to assign a name to the fungus, he discussed its similarity to "*P. entospora*," cited *P. "Kellermani"* as a synonym, mentioned finding oospores, and stated the host. Saccardo's description is obviously based on Swingle's discussion. Nevertheless, Saccardo was the first to validly publish a name for this downy mildew on *Iva xanthifolia* Nutt. If a shorter citation is desired, *B. Kellermanii* (Sacc.) Wilson is in conformity with the Rules. Saccardo, being the validly publishing author, must not be omitted.

2. **Bremia ganglioniformis** (Casp.) n. comb.—Usually cited as *B. Lactucae* Regel.

Bremia Lactucae Regel in Bot. Zeit. **1**: 666. T. 3B, fig. 1. 1843.
Botrytis ganglioniformis Berk. in Jour. Hort. Soc. Lond. **1**: 31. T. 4, fig. 25. 1846.
Peronospora ganglioniformis (Berk.) Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores observed; inadequately described.)
Peronospora ganglioniformis Tul. ex Caspary in Monatsb. K. Preuss. Akad. Wiss. **1855**: 330. 1855. (Reference made to both Berkeley and Tulasne.)
Peronospora gangliformis Berk. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 106. T. 8, fig. 1–3. 1863. (Oospores.)
Bremia Lactucae Regel ex Schroeter in Cohn, Krypt. Fl. Schles. **3** (1): 239. 1886. (Oospores.)

Regel, in originally describing and illustrating the fungus, knew of only the imperfect stage. Although Tulasne was the first to observe the oospores, he stated merely that they were smooth. Since the fungus cannot be identified on this character alone, his combination must be considered a *nomen nudum*. Caspary, by referring to both Tulasne's paper and Berkeley's, fulfilled the requirements for valid publication.

Subsequent to Regel, Schroeter was the first to use the generic name *Bremia* again. He described the oospores in his generic description; the genus should therefore be cited *Bremia* Regel ex Schroeter, or, for brevity, *Bremia* Schroeter.

The above is one example of the type of changes that must be made if one applies Article 57 to the Phycomycetes. This case presents a strong argument in favor of Bisby's proposed change in Article 57. *Bremia Lactucae* Regel, a binomial well established in mycological and phytopathological literature, would be preserved and the new combination would not be necessary. Furthermore, it seems illogical to base the binomial on the perfect stage, when the fungus cannot be positively identified if only that stage is present. The imperfect stage, on the other hand, is diagnostic, and it alone serves to readily identify the fungus.

3. *Peronospora alta* Farlow—Usually cited as *Per. alta* Fuckel.

Peronospora alta Fuckel, Fungi rhen. No. 39. 1863. (Also Hedw. **2**: 133. 1863.)
Peronospora alta Fuckel ex Farlow in Bot. Gaz. **8**: 328. 1883. (Oospores.)

According to European workers, the oospores of *Peronospora alta* are rarely observed (**7**). Farlow, in 1876 (**5**), was apparently the first to observe them and stated that they were especially abundant. However, since Farlow incorrectly identified the fungus as *Per. effusa* in this work, the reference given above for oospores is to his later paper in which the name *Per. alta* is used.

4. *Peronospora Plantaginis* Burrill apud Underwood emend.—Usually cited as *Per. Plantaginis* Burrill.

Peronospora Plantaginis Burrill apud Underwood in Bul. Torrey Bot. Club **24**: 83. 1897.

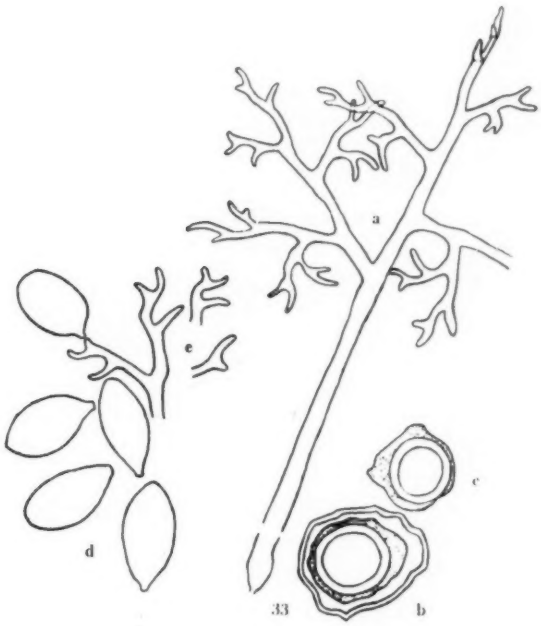

FIG. 1. *Peronospora Plantaginis*. a, d, e, on *Plantago aristata;* b, c, on *Plantago Purshii*.

Foliicolous; infected area light brown, extending the width of the leaf, appearing unoccupied below due to the sparseness of the mat and the pubescence of the host; conidiophores hypophyllous, aseptate, hyaline, emerging singly or in groups of 2–5 from the stomata, 4–6 times monopodially branched, 300–790 × 7.5–12 μ; crown 1/3 or less the total height, main branches straight or nearly

so; ultimate branches acute, stout, unequal, curved, 4–15 μ long, the axial longer, occasionally sigmoid; conidia light brown to violaceous, elliptical to ellipsoid, somewhat pointed apically, distinctly pointed basally, often with a basal projection, 31–43 × 15–23 μ (Mean 34.37 × 19.21 μ); oogonia 38–60 μ diam., wall approximately 2.5 μ thick, dark yellow-brown; oospores 30–54 μ diam., exospore at most light yellow-brown, often unilaterally thickened up to 7 μ, externally uneven; endospore 2–4 μ thick, hyaline (FIG. 1).

On Plantaginaceae: Oospores and conidia on: *Plantago Purshii* R. & S. (Collected June 21, 1943. Madison, Dane Co., Wisc. H. C. Greene. Specimen deposited in the Cryptogamic Herbarium of the University of Wisconsin.) Conidia on: *Plantago aristata* Michx.

Range: Ala. to N. Car. and northward to southern Wisc.

As pointed out by Wilson (**11**), both *Peronospora Plantaginis* and *Per. alta* occur on members of the Plantaginaceae. The conidiophores of *Per. Plantaginis* exhibit a more nearly monopodial type of branching and are stouter; the main branches are straighter, and the ultimate branches are shorter and stouter. The conidia of *Per. Plantaginis* are somewhat larger (not smaller, as Wilson (**11**) states; see original description), are distinctly pointed basally and somewhat so apically. The oogonia of the two species are also distinct, those of *Per. alta* having thin, hyaline walls, while those of *Per. Plantaginis* have thick, dark yellow walls. *Per. Plantagnis* is therefore placed in de Bary's section Parasiticae, while *Per. alta* belongs in section Effusae.

The identity of the oospores noted by Wilson (**11**) in *Plantago pusilla* Nutt. remains unsettled, since they are much larger than those of either *Per. alta* or *Per. Plantaginis*. Their diameter, as reported by Wilson ("60–95 μ across"), is above the size usually encountered in members of the Peronosporaceae (**4**).

5. *Peronospora candida* de Bary—Usually cited as *Per. candida* Fuckel.

Peronospora candida Fuckel, Fungi rhen. No. 38. 1863. (Also Hedw. **2**: 132. 1863.)

Peronospora candida Fuckel ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 120. 1863. (Oospores.)

6. *Peronospora Alsinearum* de Bary—Usually cited as *Per. Alsinearum* Caspary.

Peronospora Alsinearum Caspary in Monatsb. K. Preuss. Akad. Wiss. **1855**: 330. 1855.
Peronospora Alsinearum Caspary ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 113. T. 8, fig. 9–18. 1863. (Oospores.)

7. *Peronospora effusa* de Bary—Usually cited as *Per. effusa;* the author citation varies.

Botrytis effusa Grev., Fl. Edin. p. 468. 1824. (Pre-Friesian.)
Botrytis farinosa Fries, Sys. Myc. **3**: 404. 1832.
Botrytis effusa Grév. ex Desmazières in Ann. des Sci. Nat., Ser. II, **8**: 5. T. 1, fig. 1. 1837.
Peronospora farinosa Fr., Sum. Veg. Scand. p. 493. 1849.
Peronospora effusa Ces. in Rabenh., Herb. Myc., ed. I, No. 1880. 1854. (Also in Bot. Zeit. **12**: 190. 1854.)
Peronospora effusa (Grev.) Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores; but inadequately described. Nomen nudum.)
Peronospora effusa Grev. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 115. T. 8, fig. 7; T. 13, fig. 11. 1863. (Oospores.)

Here is an instance where the adoption of Bisby's proposal would defeat "established custom," as the binomial *Peronospora effusa* de Bary would have to be replaced with *Per. farinosa* (Fries) Fries, a combination evidently overlooked by both Keissler and Wilson (**11**). Following Bisby's proposal, even if one recognizes several species of downy mildews on chenopodiaceous hosts, the epithet "farinosa" would have to be maintained for one of them, since that name was employed by Fries in his *Systema.*

8. *Peronospora Ficariae* de Bary—Usually cited as *Per. Ficariae* Tulasne.

Peronospora Ficariae Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores observed; inadequately described. Nomen nudum.)
Peronospora Ficariae Tulasne ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 117. 1863. (Oospores.)

Mere mention of the occurrence of oospores does not constitute a description of the fungus. Tulasne was the first to find oospores of this and other species of *Peronospora.* He briefly characterized the oospores of seven of them, but did not write descriptions. He failed to indicate the hosts and he did not refer to descriptions by

other authors. I see no alternative, therefore, but to consider Tulasne's binomials *nomina nuda*.

9. *Peronospora grisea* de Bary—Usually cited as *Per. grisea* (Unger) Unger.

Botrytis grisea Unger, Exantheme d. Pfl. p. 172. 1833.
Peronospora grisea Ung. in Bot. Zeit. **5**: 315. 1847.
Peronospora grisea Unger ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 119. T. 13, fig. 12. 1863. (Oospores.)

10. *Peronospora obovata* de Bary—Usually cited as *Per. obovata* Bonorden.

Peronospora obovata Bon. in Rabenh., Fungi europ. II, No. 289. 1860. (Also Bot. Zeit. **19**: 104. 1861.)
Peronospora obovata Bonorden in Rabenh. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 121. 1863. (Oospores.)

11. *Peronospora parasitica* de Bary—Usually cited as *Per. parasitica* (Pers.) Tul.

Botrytis parasitica Persoon, Obs. Myc. **1**: 96. T. 5, fig. 6. 1796. (Pre-Friesian.)
Botrytis parasitica Pers. ex Fries, Sys. Myc. **3**: 404. 1832.
Peronospora parasitica Fr., Sum. Veg. Scand. p. 493. 1849.
Peronospora parasitica (Pers.) Tulasne in Compt. Rend. Acad. Sci. **38**: 1103. 1854. (Oospores observed; inadequately described.)
Peronospora parasitica Pers. ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 117. 1863. (Oospores.)

According to the present wording of Article 57, the proper citation is simply *Peronospora parasitica* de Bary. If Bisby's proposal is followed, the citation becomes *Per. parasitica* (Pers. ex Fr.) Fr. The volume in which Fries published this combination, and others, evidently has long been overlooked by taxonomists of the Peronosporaceae.

12. *Peronospora Potentillae* Farlow—Usually cited as *Per. Potentillae* de Bary.

Peronospora Potentillae de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 124. 1863.
Peronospora Potentillae de Bary ex Farlow in Bot. Gaz. **8**: 314. 1883. (Oospores.)

13. *Peronospora Schleideniana* Cornu—Usually cited as *Per. destructor*; the author citation varies.

Botrytis destructor Berk. in Ann. Mag. Nat. Hist. I, **6**: 436. T. 13, fig. 23. 1841.
Peronospora Schleideni Ung. in Bot. Zeit. **5**: 315. 1847.
Peronospora destructor Fr., Sum. Veg. Scand. p. 493. 1849.
Peronospora Schleideniana Unger ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 122. 1863.
Peronospora Schleideniana Unger ex Cornu in Bul. Soc. Bot. France **25**: 298. 1878. (Oospores.)

The nomenclature of the organism causing downy mildew of onion has been discussed by several workers, including Yarwood (**12**), Wakefield and Moore (**10**), Gäumann (**7**), and Wilson (**11**). Wakefield and Moore, in attempting to follow the present wording of Art. 57, credit G. W. Smith with first finding the oospores of the fungus; they evidently overlooked Cornu's earlier description. Since Cornu is the validating author, the spelling used by him for the specific epithet is correct, rather than Unger's original spelling. If Bisby's proposal is followed, the binomial now in common use is the proper one, the correct author citation being *Per. destructor* (Berk.) Fries.

14. *Peronospora Urticae* de Bary—Usually cited as *Per. deBaryi* Salm. & Ware.

Peronospora Urticae (Lib.) de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 117. 1863. (Oospores.) (Synon. exclusis.)
Peronospora deBaryi Salm. & Ware in Trans. Brit. Mycol. Soc. **14**: 47; figs. 1–6. 1929. (Oospores.)

15. *Pseudoperonospora Urticae* Salm. & Ware—Usually cited as *Pseudoper. Urticae* (Lib.) Salm. & Ware.

Botrytis Urticae Lib. ex Berk. in Jour. Hort. Soc. Lond. **1**: 31. 1846. (Nomen nudum.)
Botrytis Urticae Lib. ex Berk. & Broome in Ann. Mag. Nat. Hist., Ser. II, **7**: 100. 1851.
Pseudoperonospora Urticae (Lib.) Salm. & Ware in Trans. Brit. Mycol. Soc. **14**: 47. 1929. (Oospores.)

In a recent paper (**9**) I discussed the history of the two downy mildew species occurring on *Urtica* spp. and pointed out that it is *Peronospora deBaryi* Salm. and Ware which occurs in the U. S. In that paper the nomenclature employed by Salmon and Ware was used. If Bisby's proposal is followed, the nomenclature employed by Salmon and Ware is essentially correct. I should prefer, how-

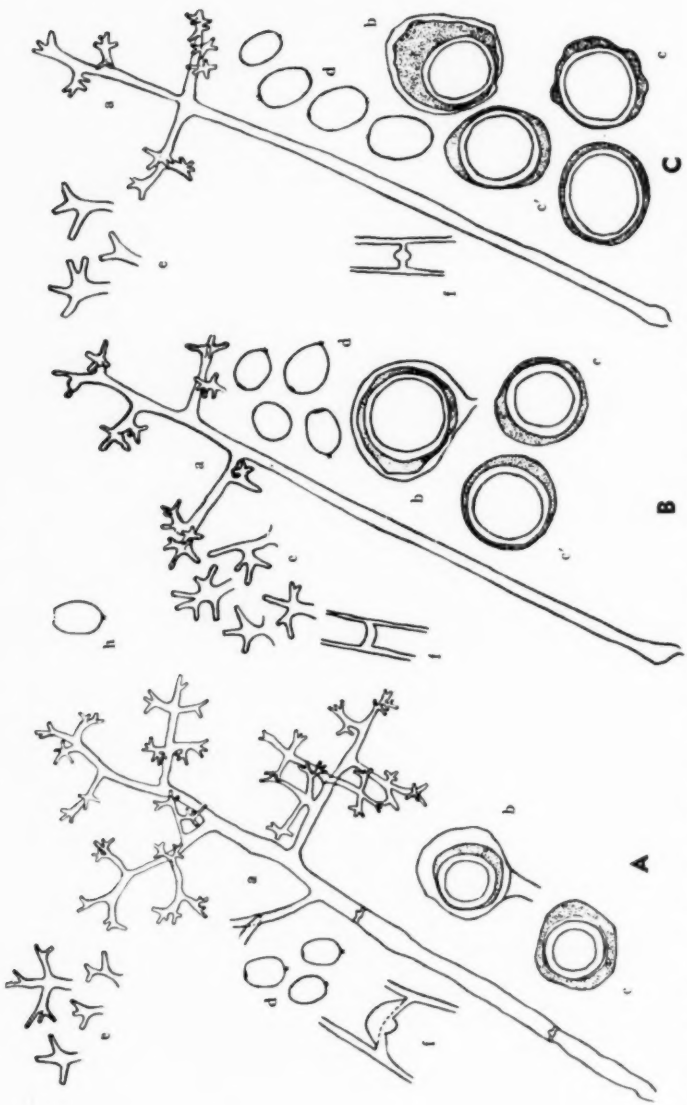

FIG. 2. A. *Plasmopara Acalyphae* on *Acalypha virginica*. B. *Plasmopara ribicola* on *Ribes triste*. C. *Plasmopara Viburni* on *Viburnum Opulus* var. *americana*.

ever, the citation *Pseudoperonospora Urticae* (Berk. & Broome) Salm. & Ware, since Berkeley and Broome must be considered the first to have validly published a binomial for the downy mildew occurring on *Urtica* and producing apically papillate, poroid conidia.

If Article 57, as now worded, is followed, the *Peronospora* becomes *Per. Urticae* de Bary, and the *Pseudoperonospora* becomes *Pseudoper. Urticae* Salm. & Ware. No other author should be be cited for either.

16. *Plasmopara Acalyphae* Wilson emend.—Usually cited as *Plas. Acalyphae* Wilson.

Plasmopara Acalyphae Wilson in Mycologia **10**: 169. 1918.

Foliicolous; infected area small, up to 1.0 cm. across, irregular in shape, at first chlorotic, becoming light brown, finally dark brown with a reddish margin; white, usually sparse, mat below; conidiophores hypophyllous, septate, hyaline, emerging from the stomata singly or in groups of 2–3, monopodially branched 4–5 (6) times, 280–570 × 5–10 μ; crown $\frac{1}{3}$ to $\frac{1}{2}$ the total height, loosely branched, primary branches 4–7 in number, straight, arising at acute to right angles; ultimate branches 2–5 in number, slightly conic, straight, narrow, truncate, very short, mostly 3–5 μ, a few up to 12 μ long; conidia hyaline, contents occasionally chlorinous, broadly ovate to subglobose, basally pedicellate, apically indistinctly poroid, 11–16 × 9–14 μ (Mean 13.91 × 11.10 μ); oogonia 33–44 μ diam., wall thin, yellowish; oospores small, 27–38 μ diam., exospore light yellowish, externally smooth, often thickened unilaterally up to 5 μ; endospore hyaline, 3–4 μ thick (FIG. 2, A).

On Euphorbiaceae: Oospores and conidia on: *Acalypha virginica* L. (Collected Oct. 14, 1926. Blue River, Grant Co., Wisc. J. J. Davis. Specimen deposited in the Cryptogamic Herbarium of the University of Wisconsin.)

Range: Wisconsin.

This is believed to be the first description of the oospores.

17. *Plasmopara Geranii* (Farlow) Berlese & De Toni—Usually cited as *Plas. Geranii* (Peck) Berlese & De Toni.

Peronospora Geranii Peck in Rept. N. Y. State Mus. Nat. Hist. **28**: 63. 1876.

Peronospora Geranii Peck ex Farlow in Bot. Gaz. **8**: 311. 1883. (Oospores.)

Plasmopara Geranii (Peck) Berl. et De Ton. in Sacc., Syll. Fung. **7**: 242. 1888. (Oospores.)

18. **Plasmopara Hepaticae** (Caspary) n. comb.—Usually cited as *Plas. pygmaea* (Unger) Schroeter.

Botrytis pygmaea Unger, Exantheme d. Pfl. p. 172. 1833.
Peronospora pygmaea Ung. in Bot. Zeit. **5**: 315. T. 6, fig. 8. 1847.
Peronospora Hepaticae Caspary in Monatsb. K. Preuss. Akad. Wiss. **1855**: 329. T. 6, figs. 22–26. 1855. (Oospores.)
Plasmopara pygmaea (Unger) Schroeter in Cohn, Krypt. Fl. Schles. **3** (1): 239. 1886. (Oospores.)

Caspary was the first to describe the oospores of this species. Both "acrospores" (conidia) and "sporangia" (oogonia) were described and figured for his *Peronospora Hepaticae*. According to the present wording of Article 57, *Plasmopara Hepaticae* (Casp.) n. comb. is correct, rather than *Plas. pygmaea* (Ung.) Schroet., which would be correct according to Bisby's proposal and which is in common use.

19. *Plasmopara Illinoensis* Davis—Usually cited as *Plas. Illinoensis* (Farlow) Davis.

Peronospora Illinoensis Farlow in Bot. Gaz. **8**: 332. 1883.
Plasmopara illinoensis (Farl.) Davis in Trans. Wis. Acad. Sci. **21**: 280. 1924. (Oospores.)

20. *Plasmopara nivea* (de Bary) Schroeter—Usually cited as *Plas. nivea* (Unger) Schroeter.

Botrytis nivea Martius ex Unger, Exantheme d. Pfl. p. 171. T. 2, fig. 14. 1833. (Not *B. nivea* Martius, Fl. Erlang. 1817.)
Peronospora nivea Ung. in Bot. Zeit. **5**: 314. 1847. (Pro parte.)
Peronospora nivea Unger ex de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 105. T. 4. 1863. (Oospores.)
Plasmopara nivea (Unger) Schroeter in Cohn, Krypt. Fl. Schles. **3** (1): 237. 1886. (Oospores.)

21. *Plasmopara ribicola* Davis emend.—Usually cited as *Plas. ribicola* (Schroeter) Schroeter.

Peronospora ribicola Schroeter in Jahresber. d. Schles. Gesellsch. Vaterl. Kult. **61**: 179. 1883. (Fide Schroeter, 1886.)
Plasmopara ribicola (Schroeter) Schroeter in Cohn, Krypt. Fl. Schles. **3** (1): 238. 1886.
Plasmopara ribicola Schroet. ex Davis in Trans. Wis. Acad. Sci. **18**: 94, 251. 1915. (Oospores.)

In his 1886 publication, Schroeter gives the following reference as the place of publication of his *Peronospora ribicola:* "Jahresber. d. Schles. Gesellsch. f. 1883. S. 179." Examination of this publication disclosed that the cited page is included in an article by Schroeter in which four new species of Peziza are described on "S. 179." No mention of a downy mildew could be found anywhere in the article, or in the entire volume.

Davis first described the oospores of *Plasmopara ribicola,* but failed to mention the hosts or the conidial stage, or to give any reference to a previous description. He stated that the oogonial wall is symmetrically thickened on two opposite sides. Actually it is the exospore wall that is so thickened, not the oogonial wall. Such lateral thickening is characteristic of the oospores of several species of *Plasmopara.* Since the description of the perfect stage, as published by Davis, was slightly inaccurate, and since a combined description of the oospores and the conidia of this fungus has not been published, a complete description is given below.

Foliicolous; infected area angular, limited by the veins, tan, gray-brown to dark brown or often reddish above; white, thin, loose mat below; conidiophores hypophyllous, septate, hyaline, fasciculate, 2–10 emerging from a stoma, (2) 3–5 times monopodially branched, 160–500 × 6–9 μ, base often bulbous, up to 11 μ; crown $\frac{1}{3}$ to at most $\frac{1}{2}$ the total height, main branches 2–6 in number, arising at approximately right angles, straight; ultimate branches 2–5 in number, narrowly conic, straight to slightly tortuous, narrowly truncate, mostly 4–14 μ long; conidia hyaline, broadly ellipsoid to subglobose, basal pedicel short, often indistinct; apically flatly poroid, variable in size, 11–32 × 10–22 μ (Mean 16.97 × 14.10 μ); oogonia 29–43 μ diam. wall thin, light yellow brown; oospores 26–40 μ diam., exospore hyaline when expressed, externally smooth, often thickened unilaterally or on two opposite sides up to 9 μ; endospore hyaline, 2–3 μ thick (FIG. 2, B).

On Saxifragaceae: Oospores and conidia on *Ribes Cynosbati* L., *R. gracile* Michx., *R. oxycanthoides* L., *R. prostratum* L'Her., and *R. triste* Pall. (Oospores occur in the following specimen which has received wide distribution: Fungi Columbiani No. 1753. On *Ribes rubrum subglandulosum* (= *R. triste* Pall.), Vilas Co., Wisc., July 29, 1902, J. J. Davis.)

Range: W. Va., westward to Wash.; also Europe.

23. *Plasmopara Viburni* Davis—Usually cited as *Plas. Viburni* Peck.

Plasmopara Viburni Peck in Rept. N. Y. State Mus. Nat. His. **43**: 28. 1890.

Plasmopara viburni Pk. ex Davis in Trans. Wis. Acad. Sci. **18**: 101. 1915. (Oospores.)

In describing the perfect stage of this species, Davis again characterized the oogonial wall as "often irregularly thickened." As in *Plasmopara ribicola*, I consider the exospore wall to be the structure laterally thickened. Since Davis mentioned the host and the occurrence of conidia, his description is accepted as valid. A complete description, however, is given below.

Foliicolous; infected areas angular, often located along the main veins and limited by the smaller veins, coalescing into large areas, at first watersoaked, becoming dark brown above; white, sparse, thin mat below; conidiophores hypophyllous, septate, hyaline or with light yellowish contents, up to 8 emerging from a stoma, 2–4 times monopodially branched, 120–440 × 4–9 μ, slightly bulbous basally; crown ⅓ to at most ½ the total height, main branches 2–5 in number, arising at right angles, straight or slightly tortuous, truncate, 4–17 μ long; conidia hyaline or with yellowish contents, broadly ovate, basally pedicellate, indistinctly poroid apically, variable in size, 13–34 × 11–23 μ (Mean 19.80 × 14.82 μ); oogonia 34–46 μ diam., wall thin, hyaline to light yellow; oospores 32–38 μ diam., exospore yellowish, usually appearing thin, slightly wrinkled externally, thickened laterally to 4.5 μ (occasionally to 10 μ); endospore hyaline, 3–4 μ thick (FIG. 2, C).

On Caprifoliaceae: Oospores and conidia on *Viburnum Opulus* L. var. *americanum* (Mill.) Ait. (Oospores occur in the following specimen which has received wide distribution: Fungi Columbiani No. 4280. On *Viburnum Opulus* L. var. *americanum* (Mill.) Ait. Wausaukee, Wisc. Aug. 23, 1913. J. J. Davis.)

Range: N. Y. westward to Wisc., southward to Ala.

24. *Plasmopara viticola* (de Bary) Berl. & De Toni—Usually cited as *Plas. viticola* (Berk. & Curt.) Berl. & De Toni.

Botrytis viticola B. & C. ex Berkeley in Jour. Hor. Soc. Lond. **6**: 289. 1851. (Nomen nudum.)

Peronospora viticola (Berkl. et Curtis) Caspary in Monatsb. K. Preuss. Akad. **1855**: 331. 1855. (Nomen nudum.)

Peronospora viticola (Berk. et Curt.) de Bary in Ann. des Sci. Nat., Ser. IV, **20**: 125. 1863. (Oospores.)

Plasmopara viticola (Berk. et Curt.) Berl. et De Ton. in Sacc., Syll. Fung. **7**: 239. 1888.

Since Berkeley did not publish a description, it would seem to me that the correct citation is *Plas. viticola* (de Bary) Berl. & de Toni, both according to Article 57 and to Bisby's proposal concerning Art. 57, referred to above.

The examples above show that, regardless of whether the present wording of Article 57 or Bisby's proposal is followed, some changes in the binomials and author citations are necessary for certain species of the Peronosporaceae. Fewer changes in binomials would have to be made if Bisby's proposal were accepted than if Article 57 is strictly applied. Regardless of which procedure is followed, author citations in common use for several species are incorrect.

I am in favor of changing the wording of Article 57 so that its principle will not apply to the Phycomycetes. But, until that change is actually and validly made, the only justifiable procedure is to consider that Article 57 does apply to the Phycomycetes.

Problems relating to fungus nomenclature should be thoroughly discussed in the time remaining so that definite proposals can be presented to the Congress to be held in Stockholm in 1950. So that final decisions may be reached at that time, serious thought should be given to changing the provisions of Article 74, which prescribes the method by which the Rules may be altered. Considering the long intervals of time that have elapsed between previous congresses, the provisions of this article actually defeat the very purpose of the Rules, namely stability in nomenclature.

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EXPLANATION OF FIGURES

All figures drawn with the aid of a camera lucida. a, Conidiophore × 275. b, Oogonium with enclosed oospore; drawn in optical sectional view × 325. c, Oospore(s). The exospore has been stippled in all oospore and oogonial drawings as an aid to distinguishing the various structures. Oospores drawn as they appear in the oogonium are indicated by the letter "c"; expressed oospores, by "c'" × 325. d, Conidia × 325. e, Ultimate branches × 325. f, Septation × 900. h, Germinated conidium × 325.

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A NEW SPECIES OF ACHLYA

HELEN SIMPSON REISCHER

(WITH 5 FIGURES)

In the spring of 1947 a series of collections of damp soil were made in Palisades Interstate Park, New Jersey, for the purpose of obtaining material for a comparative physiological study of the Saprolegniaceae. One of these collections proved to contain an *Achlya* which apparently has not previously been described, and for which the following name is proposed:

Achlya Sparrowii [1] sp. nov.

Mycelium in semine *Cannabis sativae* densum; cultura usque ad 1.5 cm. diam., hyphis primariis basi 60–80 μ diam. Sporangia rara, proliferantia ut in *Achlya* soleat. Exitus zoosporarum typicus generis; sporae hibernantes ca. 11 μ diam. Gemmae non formatae. Oogonia globosa, numerosa, plerumque 57 ad 77 μ diam., in ramulis lateralibus lata, cum tunica crassa, omnino levia et non punctulata. Ova 1–11, fere 3 vel 4 in oogonio quoque, plurimum 28.6–33 μ diam., maturitate subcentrica. Antheridia 1–4 in omni oogonio, origine androgyna, numquam ramosa, ad oogonium acumine applicata.

Mycelial growth dense, reaching a diameter of approximately 1.5 cm. on hemp seed. Main hyphae about 60 to 80 μ wide at the base. Sporangia somewhat rare, those formed 220–400 μ by 26–35 μ. Secondary sporangia produced by cymose branching from below. Zoospore discharge as typical for the genus, the encysted zoospores about 11 μ in diameter. Gemmae not formed in our cultures. Oogonia spheric, abundant, 33 to 101 μ in diameter, usually 57 to 77 μ, borne on short lateral stalks from the main hyphae, occasionally terminal on the main hyphae. Oogonial wall smooth, unpitted except under the antheridia. Oospores spheric, 1 to 11 in an oogonium, usually 3 or 4, mostly 28.6 to 33 μ in diameter, at maturity slightly subcentric with a circle (as seen in optical cross section) of small oil droplets, slightly thicker on one side, surrounding the protoplasm. Oospore wall smooth, thick. Antheridia on all oogonia, 1–4 per oogonium, arising from the oogonial

[1] This species is named in honor of Professor F. K. Sparrow, Jr.

stalk near the neck of the oogonium, from the neck of the oogonium, or less often from its spherical surface. Antheridia simple, clavate, always applied by the tip, never laterally applied and never branching. Fertilization tubes visible.

Type locality: Palisades Interstate Park, New Jersey.

The description given above was drawn from unifungal cultures less than five days old, grown on hemp seed, which has become the standard substratum for members of the Saprolegniaceae, in charcoal-treated distilled water in petri dishes, at 20° C. *A. Sparrowii*, in these cultures, showed a marked resemblance to *A. racemosa* in its smooth oogonia, nearly centric oospores, and the general appearance of the androgynous antheridia. The oospores of *A. racemosa* are, however, sufficiently smaller to prevent confusion of the two species. Older cultures on hemp seed showed affinities to *A. polyandra*, in which the oospores are only slightly smaller than in our fungus, by an increase in the usual number of oospores in an oogonium and in the number and length of the antheridial branches. These hemp-seed cultures, though regularly washed, contained various unidentified bacterial contaminants. It seems reasonable to suppose that, among other factors, available food and oxygen vary considerably in different parts of the culture and in time.

In order to determine the degree of variation in the important sexual characters, which could be produced by changing the method of culture, *Achlya Sparrowii* was also grown in pure culture on (Difco) corn meal agar with 1 g./liter of yeast extract added and in pure culture in 20 ml. portions (in 250 cc. Erlenmeyer flasks) of a liquid medium consisting of 5 g./liter yellow corn meal (extracted by boiling for ½ hour and centrifuged clear before use) and 1 g./liter of yeast extract, at temperatures ranging from 10 to 30° C. at 5 degree intervals. The mycelial mats grown in the liquid medium were transferred to sterile charcoal-treated distilled water in petri dishes immediately after the formation of the first oogonial initials (after 3 to 5 days, depending upon the temperature). No growth occurred at 30° C. under any circumstances. Growth at 10° C. was quite slow, and the oogonia and oospores formed (on agar and hemp seed) frequently aborted. No further use, therefore, was made of these temperatures.

Temperature had no obvious effect upon the antheridia; the medium employed, and the age of the culture (in hemp-seed cultures only), affected the number, origin, size and shape, and the degree of branching of the antheridial stalks. In young cultures on hemp seed the antheridia were 1–4, usually 2, to an oogonium, a slight majority arising from the neck of the oogonium, nearly as many from the stalk just below the oogonial neck, a few from the spherical surface of the oogonium. No branching of the antheridial stalks was observed. Cultures on agar exhibited substantially the same characteristics. In hemp-seed cultures over five days old, however, slightly more of the antheridia arise from the oogonial stalk; the antheridial branches, usually about 25 μ in length in the younger cultures and those on agar, become elongated loops up to 250 μ in length, exclusive of the antheridium, and the antheridia, clavate in younger cultures, become tubular, with a diameter slightly less than that of the antheridial stalk. There may be, though rarely are, as many as eight antheridia applied to a single oogonium and, *very rarely*, the antheridial stalks may branch, though never the antheridia. Mats grown in the liquid medium described produce antheridia resembling those of the older hemp-seed cultures but showing even greater tendency to arise from the oogonial stalk. The author regards this as an indication that the change from an *A. racemosa* (as figured by Coker, **1923**, pl. 31, f. 9) type of antheridial branch to an *A. polyandra* (as figured by Hildebrand, **1867**, pl. 16, f. 7–11) type *may* be caused by the low concentration of nutrient materials in the older hemp-seed cultures. It is unfortunate that more attention has not been paid to the effect of such environmental factors upon taxonomically critical characteristics. The characters remaining constant throughout our cultures were the androgynous origin of the antheridia, the lack of branching in the antheridia themselves, and the terminal application of the antheridia to the oogonium. Since the branching of the antheridial stalks of *A. polyandra* is emphasized in all published descriptions of this species, and *A. polyandra* is figured with laterally applied antheridia (Hildebrand, **1867**), these characters separate this closely related species from *A. Sparrowii*.

The diameter of the oogonium is closely correlated with the number and diameter of the oospores formed and is therefore of

minor importance as a separate character, varying from 33 μ (one oospore) to 101 μ (11 oospores), usually 57 to 77 μ (2 to 4 oospores) in the younger cultures on hemp seed.

Temperatures from 15 to 25° C. had no apparent effect on the number of oospores formed in the hemp-seed cultures. Three or four oospores per oogonium were usually produced in the younger cultures, 6 in the older cultures. In cultures on agar the oospore number did not vary with time but did vary slightly with temperature: mostly 2–3 oospores at 25° C., 3 at 20° C., and 3–4 at 15° C. Since a difference of 1 or 2 in the usual number of oospores has not been used to distinguish species, except where only one oospore is typically produced, a detailed statistical study was not considered necessary. Oogonia produced by mats grown on liquid media usually contained only 1 or 2 oospores, a probable result of lack of food materials, as similar results are obtained by placing agar discs containing mycelium in water at room temperature. It is noteworthy that the usual number of oospores produced does not approach the 10–15 typical of *A. polyandra*. In this respect our fungus resembles *A. racemosa* (described in Coker, **1923**, as having usually 2–5 oospores) much more closely.

In order to ascertain whether a correlation existed between the number of oospores in an oogonium, and oospore diameter, 321 oospores from hemp-seed cultures grown at 15° C. were measured. The size of the samples taken was as follows: 22 oospores from oogonia containing 1 oospore; 50 oospores each from oogonia containing 2, 3, 4, 5, and 6 oospores; 30 from oogonia containing 7; 9, 7 and 3 oospores from oogonia containing respectively 8, 9 and 10 oospores. Half of the oospores in each of the larger samples were from cultures 5 days old, half from 8 day cultures. While the diameter of the oospores reaches extremes of range in oogonia containing only 1 oospore, no significant correlation of diameter with either number of oospores or age of culture was found. The combined results (the broken line in figure 1) give a typical distribution curve of oospore diameter for *A. Sparrowii* in hemp-seed culture. In determining the effect of temperature on oospore size, measurements were made, to prevent possible bias, only on oospores (one per oogonium) in oogonia containing 3 oospores (since singly produced oospores are apt to be aberrant, and the oospores of oogonia contain-

ing only two oospores are typically flattened spheres, the true diameter of which cannot be directly measured). Diameter did not vary clearly with temperature in the cultures on hemp seed, perhaps because so many other variables are present in such impure cultures. Measurements of 30 oospores each from hemp-seed cultures grown at 15, 20 and 25° C. fell substantially on the curve for the hemp-seed cultures in figure 1. Mats grown in a liquid medium did not

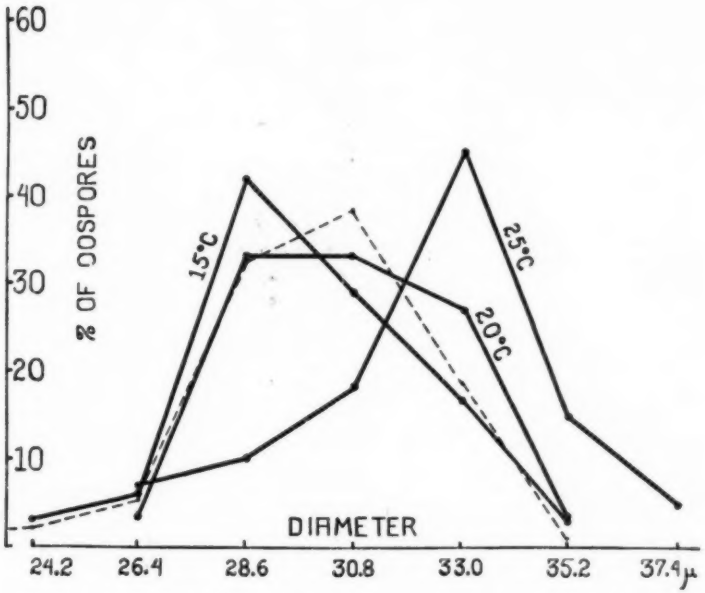
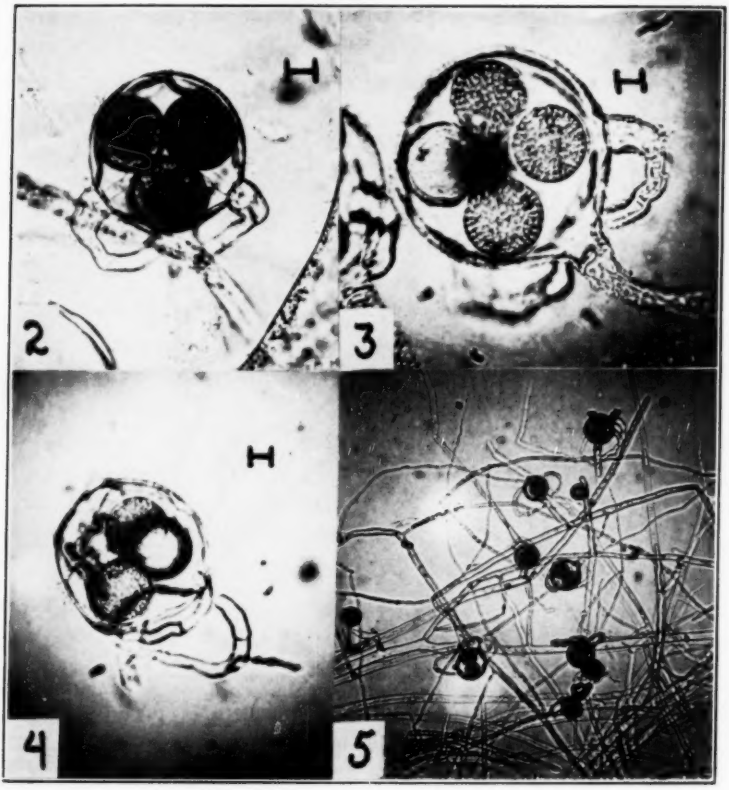


FIG. 1. The effect of temperature on oospore diameter in *Achlya Sparrowii*. Solid lines: cultures on agar grown at 15, 20 and 25° C. Broken line: hemp-seed cultures grown at 15° C.

produce oospores suitable for measurement. The variation found in cultures grown on agar is shown graphically in figure 1. The diameter of 35 oospores from cultures grown at 15° C., 30 from cultures grown at 20° C., 60 (in two samples of 30) from cultures grown at 25° C., was measured in units of 2.2 μ. The figures on the abscissa in figure 1 represent, then, the mean of a size class including 2.2 μ. The difference between the mean diameter of oospores produced at 15° C. ($29.9 \pm 0.380\ \mu$) and 25° C. ($32.3 \pm$

0.338 μ) is statistically significant. The oospores of *A. Sparrowii* are considerably larger than the "most about 22 μ" (Coker, 1923) of *A. racemosa;* no experimental treatment brought the average



FIGS. 2–5. *Achlya Sparrowii.* The photomicrographs were taken with a Leica Makam using 6 × and 10 × oculars and a 44 × objective for figures 2–4, a 10 × objective for figure 5. The lines drawn on figures 2–4 indicate the relative length of 10 μ. FIG. 2. An oogonium with 3 oospores, antheridia arising from the neck of the oogonium and the oogonial stalk. The fertilization tubes are visible. × 400. FIG. 3. An oogonium with 5 nearly mature oospores, an antheridium arising from the spherical surface of the oogonium near the neck. × 400. FIG. 4. An oogonium with 4 mature oospores, showing the slightly subcentric structure of the oospores at maturity. × 325. Figures 2–4 from hemp-seed cultures. Fig. 5. A group of hyphae from a mat grown on a liquid medium. Note the reduction of oospore number to one or two, the long looping antheridial branches. Approximately × 60.

oospore diameter as low as the 27 μ (Humphrey, 1892) of *A. polyandra*. Oospore diameter appears to be a relatively stable, and therefore extremely useful, taxonomic character.

SUMMARY

A new species of *Achlya* has been described under the name *Achlya Sparrowii*. Among the species of *Achlya* with centric or slightly subcentric oospores, androgynous antheridia and smooth oogonial walls there are only two, *A. racemosa* and *A. polyandra*, which closely resemble this fungus. *A. Sparrowii* may be distinguished from *A. racemosa* quite readily by its much larger oospores, from *A. polyandra* by its slightly larger, fewer oospores and the usually fewer, only rarely branched antheridial stalks with antheridia applied to the oogonia by the tip rather than laterally. The effect of growth in pure culture on liquid and solid media and of common laboratory temperatures on these characters has been described.

COLUMBIA UNIVERSITY AND THE NEW YORK BOTANICAL GARDEN,
NEW YORK, N. Y.

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TWO SPECIES REPRESENTING A NEW GENUS OF THE CHAETOMIACEAE

R. K. BENJAMIN [1]

(WITH 33 FIGURES)

In the course of a study of some coprophilous fungi, the author found what appears to be an undescribed ascomycete resembling in certain fundamental features fungi placed in the Chaetomiaceae. The first perithecia were found growing on goat dung and mass spore inoculations from these were made on potato dextrose agar and Blakeslee's No. 230 but on neither media were perithecia produced or anything other than sterile mycelia, even after two months. Transfers were made to dung agar and on this medium perithecia matured normally in less than three weeks and were identical to those which were found growing naturally on goat dung.

While attending the Chicago meetings of the Mycological Society of America in December 1947, the author mentioned to Professor G. W. Martin the isolates that he had been studying and Professor Martin indicated immediately that he had obtained a very similar fungus from a collection made in Peru in 1945. Professor Martin later very kindly sent cultures of his isolate for study. It was obvious from the first that the two organisms were congeneric but specifically distinct. These have been studied side by side on various types of media and their characteristics and differences noted. The characteristics of the perithecial walls, the evanescent asci and light-colored spores, the appendages surrounding the ostiole of the perithecium, and the manner in which the ascospores are discharged in the form of an elongate cirrus suggest a relationship to the genus *Chaetomium*. An examination of the monographs by Bainier (**1**), Palliser (**4**), and Chivers (**2**), and the more recent papers by Greathouse and Ames (**3**), Tschudy (**5**) and others has indicated that neither of these species could be as-

[1] The encouragement and help of Professor Leland Shanor during this study is gratefully acknowledged.

signed to this genus or any related genera in the family Chaetomiaceae. Because of their long necks and submerged habit of growth these two isolates are being designated here as representing new species of a new genus.

Lophotrichus gen. nov.[2]

Perithecia immersa vel semiimmersa, globosa, collo longo et angusto, fusca et opaca, ostiolata; pariete membranaceo et fragili, cui sunt appendiculae in similitudinem pilorum varie immutatorum redactae; ascis tenuibus, delicatis, subglobosis vel late clavatis cum stirpe brevi, evanidis, octosporis, qui paraphysibus carent; sporis unicellularibus, pallidis, pomi citrei formam referentibus.

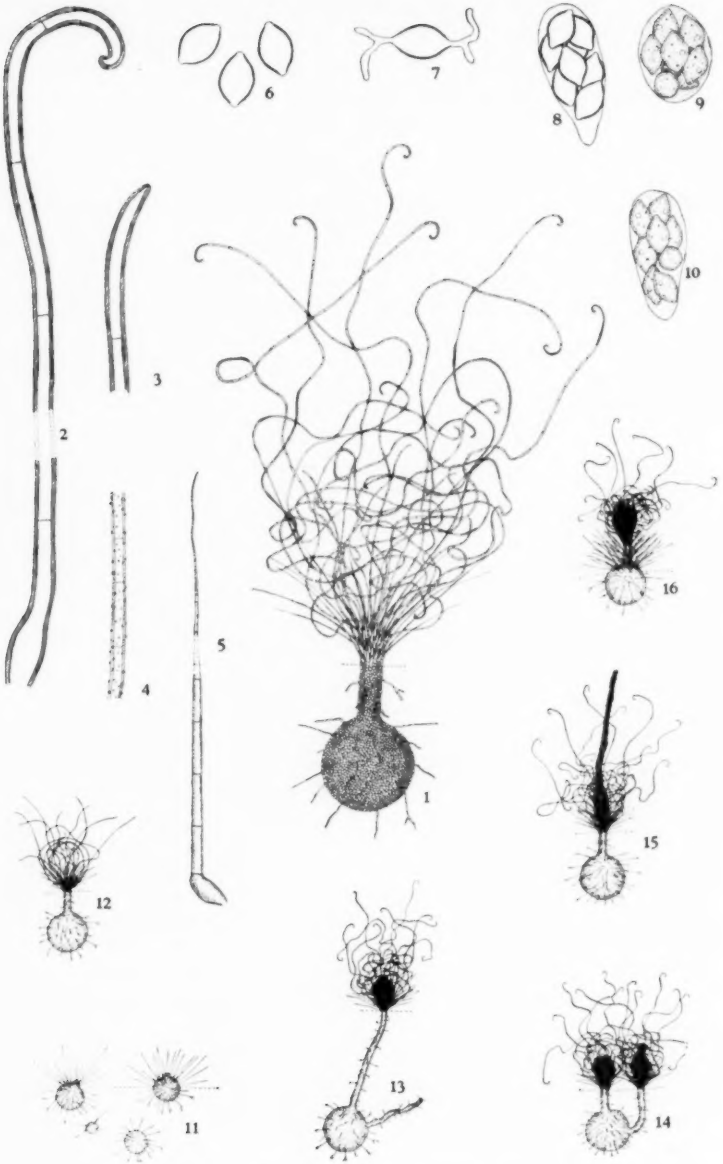
Perithecia submerged or partially superficial, spherical and translucent when young, when mature globose with a long, narrow neck, dark and opaque, tip of neck pierced by an ostiole. Perithecial wall membranaceous, brittle, provided with appendages in the form of variously modified hairs. Mycelium mostly submerged, rarely superficial. Asci thin-walled, delicate, subglobose to broadly clavate and short stalked, very evanescent, 8-spored, paraphyses lacking. Spores single-celled, light-colored, lemon-shaped.

Type species, *Lophotrichus ampullus*.

Lophotrichus ampullus sp. nov. (FIGS. 1–16)

Perithecia in fimo agaris nata, nigra, globosa, 150–260 μ in diametro (vulgo 195 μ), immersa vel semiimmersa, quorum pars immersa mycelia fusca et rhizoidea, pars superior pilos laterales formam hypharum incoloratarum, septatarum et aëriarum habentes ferunt, tenuia, membranacea; collis plerumque singulis sed interdum binis, nigris, 130–760 μ longis vel etiam longioribus, 40–60 μ in diametro; pilis terminalibus, qui ostiolum cingunt, septatis, crassis, rectis sive inaequabiliter contortis, usque ad 1.6 mm. longis, 3.8–5.3 μ in diametro (vulgo 4.3 μ), parietes 0.57–1.52 μ crassos (vulgo 1.13 μ) habentibus, fuscis et quasi fumosis, plus minusve confertim incrustatis, cacumina curva sive circinata ferentibus; ascis subglobosis vel late clavatis cum stirpe brevi, incoloratis, 10–20 $\mu \times$ 20–34 μ, evanidissimis, octosporis, qui paraphysibus carent; ascosporis, quae cum extruduntur cirrum usque ad 1.5 mm. longum saepius figurant, colorem cyprii cum glomerantur referentibus, hyalinis, pomi citrei formam habentibus, 5.3–7.6 $\mu \times$ 6.8–10.6 μ (vulgo 5.97 $\mu \times$ 8.75 μ), tenui pariete praeditis in cacuminibus, eo modo formatis ut utrubique germinent.

[2] The author is indebted to Professor Revilo Oliver, Classics Department, for the preparation of the Latin diagnoses.



FIGS. 1–16. *Lophotrichus ampullus.*

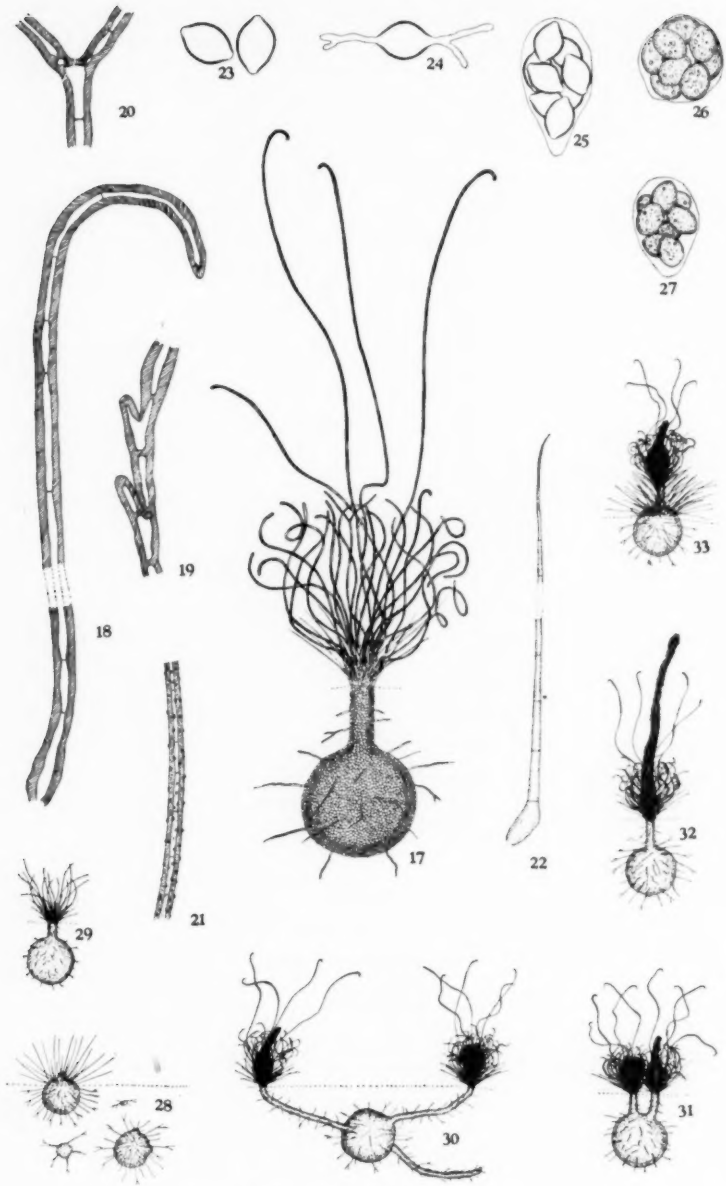
Mycelium on dung agar developing rapidly, remaining white, submerged, rarely with aerial hyphae; perithecia forming in 3–5 days, maturing in 2–3 weeks, black, globose, 150–260 μ in diameter (av. 195 μ), immersed or partially superficial, with dark, rhizoid-like mycelia on submerged parts, or lateral hairs in the form of colorless, septate, aerial hyphae on superficial portions; walls thin, membranaceous, necks usually one, occasionally two on a perithecium, black, 130–760 μ long or longer, more or less uniform in diameter, 40–60 μ; lateral hairs colorless, septate, acuminate, up to 150 μ long, 2–3 μ in diameter at base; ostiole surrounded by many long, septate, thick-walled terminal hairs, straight or irregularly contorted, up to 1.6 mm. long, 3.8–5.3 μ in diameter (av. 4.3 μ), walls 0.57–1.52 μ thick (av. 1.13 μ), dark, smoky in color, more or less densely encrusted, tips curved to circinate; asci subglobose to broadly clavate and short stalked, colorless, 10–20 μ × 20–34 μ, very evanescent, 8-spored, paraphyses lacking; ascospores extruded as a cirrus, frequently up to 1.5 mm. in length, bright copper colored in mass, hyaline, lemon-shaped, tips thin-walled, 5.3–7.6 μ × 6.8–10.6 μ (av. 5.97 μ × 8.75 μ), germinating at both ends.

Isolated from goat dung collected near Urbana, Illinois, November 1947. Herbarium material of the isolate representing the type deposited in the Mycological Collections, University of Illinois Herbarium (Mycological Collections No. 1763); the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.; Farlow Herbarium, Harvard University; and the Herbarium of the State University of Iowa, Iowa City.

Lophotrichus martinii sp. nov.[3] (FIGS. 17–33)

Perithecia in fimo agaris nata, nigra, globosa, 220–330 μ in diametro (vulgo 250 μ), immersa vel semiimmersa, quorum pars immersa mycelia fusca et rhizoidea, pars superior pilos laterales formam hypharum incoloratarum, septatarum et aeriarum habentes ferunt, tenuia, membranacea; collis plerumque singulis sed nonnunquam binis, trinis vel quaternis, nigris, 200–1000 μ longis vel etiam longioribus, 40–65 μ in diametro; pilis terminalibus, qui ostiolum cingunt, septatis, crassis, rectis sive curvis, plerumque 130–450 μ longis, quorum duo vel plures se usque ad 1.5 mm. solent porrigere, 3.8–6.1 μ in diametro (vulgo 5.2 μ), parietes 1.15–2.28 μ crassos (vulgo 1.9 μ) habentibus, fuscis et quasi fumosis, plus minusve confertim incrustatis, et cacumina recta sive curva ferentibus; ascis subglobosis vel late clavatis cum stirpe brevi, incoloratis, 11–17 μ × 20–36 μ, evanidissimis, octosporis, qui paraphysi-

[3] Named in honor of Professor G. W. Martin, who very kindly supplied this isolate for study.



FIGS. 17–33. *Lophotrichus martinii*.

bus carent; ascosporis, quae cum extruduntur cirrum usque ad 1.5 mm. longum saepius figurant, colorem cyprii cum glomerantur referentibus, hyalinis, pomi citrei formam habentibus, 5.3–6.8 $\mu \times 7$–9.9 μ (vulgo 6.4 $\mu \times 8.9$ μ), tenui pariete praeditis in cacuminibus, eo modo formatis ut utrubique germinent.

Mycelium on dung agar developing rapidly, remaining white, submerged, rarely with aerial hyphae; perithecia forming in 3–5 days, maturing in 2–3 weeks, black, globose, 220–330 μ in diameter (av. 250 μ), immersed or partially superficial, with dark, rhizoid-like mycelia on submerged parts, or lateral hairs in the form of colorless, septate, aerial hyphae on superficial portions; walls thin, membranaceous, necks usually one, commonly two to four on a perithecium, black, 200–1000 μ long or longer, more or less uniform in diameter, 40–65 μ; lateral hairs colorless, septate, acuminate, up to 150 μ long, 2–3 μ in diameter at base; ostiole surrounded by many long, septate, thick-walled terminal hairs, straight or curved, mostly 130–450 μ long with two or more commonly reaching 1.5 mm. in length, 3.8–6.1 μ in diameter (av. 5.2 μ), walls 1.15–2.28 μ thick (av. 1.9 μ), dark, smoky in color, more or less densely encrusted, tips straight or curved; asci subglobose to broadly clavate and short stalked, colorless, 11–17 $\mu \times 20$–36 μ, very evanescent, 8-spored, paraphyses lacking; ascospores extruded as a cirrus frequently 1.5 mm. in length, bright copper colored in mass, hyaline, lemon-shaped, tips thin-walled, 5.3–6.8 $\mu \times 7$–9.9 μ (av. 6.4 $\mu \times 8.9$ μ), germinating at both ends.

Isolated from dung (rabbit?) collected near Talara, Peru, September 1945, GWM No. 6290. Herbarium material of the isolate representing the type deposited in the Mycological Collections, University of Illinois Herbarium (Mycological Collections No. 1762); the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.; Farlow Herbarium, Harvard University; and the Herbarium of the State University of Iowa, Iowa City.

While these species are similar in many respects their differences are quite marked when the two are compared critically. Cultures of *Lophotrichus martinii* with numerous ascocarps appear to be distinctly darker when examined under low power magnification due largely to the greater average size of the perithecia and the much thicker walls of the terminal hairs.

When mature, a majority of the terminal hairs of *Lophotrichus ampullus* are a millimeter or more in length. Some are highly contorted so that they form a loose tangle about the ostiole while many

others are nearly straight or only slightly curved. These hairs extend upward and outward above the substrate, and have tips which are usually curved to circinate, seldom straight. By the time the ascospores have been discharged into the terminal hairs the neck has become extremely brittle slightly below the ostiole so that the least disturbance frees the mass of hairs and spores for subsequent dispersal.

The terminal hairs of *L. martinii* are distinctly different from those of the former species. The majority of them are relatively short and straight-tipped or are slightly curved to contorted, and only a few extend to the greater length common for hairs of *L. ampullus*. The walls of the hairs of *L. martinii* are much thicker than those of *L. ampullus* and less uniform in thickness throughout the length of the hair. It is not uncommon to find branching of the terminal hairs in *L. martinii* while this phenomenon has not been observed in *L. ampullus*. Spore dispersal is effected as in the latter species.

The relative abundance of perithecia possessing more than one neck may be a useful characteristic to aid in the separation of these species. This phenomenon is common to both, but differs greatly in degree. Perithecia of *L. ampullus* may develop two necks, rarely more, but this occurs in a very small percentage of ascocarps. Perithecia of *L. martinii*, on the other hand, commonly develop two to four necks, and the number of such ascocarps may reach 25 per cent or more in any given culture. While all of the necks may reach the surface of the media, develop terminal hairs and function normally, some never reach the surface. The development of terminal hairs does not occur in either speices until the tip of the neck reaches the surface of the substrate, except occassionally in very old cultures, and in such cases the pattern of development is much modified. In rare instances branching of the neck has been observed, in which case the branch may develop terminal hairs also and function in spore dispersal.

The family Chaetomiaceae has been defined to include species which characteristically form ascocarps on the surface of the substrate. Species of *Lophotrichus* typically produce perithecia partially or entirely submerged with only the ostiole and terminal hairs exposed. The production of submerged or partially submerged

perithecia is a departure from the usual habit of species of the Chaetomiaceae. When, however, all characters are taken into consideration the author believes that *Lophotrichus* is best accommodated in the Chaetomiaceae.

Lophotrichus superficially resembles also such genera as *Melanospora* and *Ceratostomella* in that it possesses an elongate neck with conspicuous hairs around the ostiole.

On the basis of the characteristics of the asci it would seem that the Chaetomiaceae, and perhaps other fungi whose asci are so very evanescent, now generally included in the Pyrenomycetes, would be more logically placed if removed to the Plectomycetes. Certainly the Chaetomiaceae and the Sordariaceae have little in common when one considers the nature of the ascus, yet these families are most frequently placed near each other in the Sphaeriales.

SUMMARY

A new genus, *Lophotrichus*, including two species, *L. ampullus* and *L. martinii*, is described, which has certain characteristics of the Chaetomiaceae. The two described species of this genus produce perithecia which, when grown in culture, are partially or entirely submerged, possess long necks, and have elongate, thick-walled terminal hairs restricted to the area immediately around the ostiole. Ascus characteristics are essentially the same as in the genus *Chaetomium*. The more or less submerged habit of the perithecia and the typical long necks are departures from the usual concepts held for the Chaetomiaceae.

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EXPLANATION OF FIGURES *

Lophotrichus ampullus. FIGS. 1–16

FIG. 1. Mature perithecium with cirrus of ascospores not shown. × 65. FIG. 2. Terminal hair with circinate tip. × 500. FIG. 3. Tip of terminal hair only slightly curved. × 500. FIG. 4. Portion of terminal hair showing encrustations. × 335. FIG. 5. Lateral hair. × 500. FIG. 6. Mature ascospores, in outline, showing thin end walls. × 835. FIG. 7. Germinating ascospore, in outline. × 835. FIG. 8. Outline of mature ascus just prior to breakdown of ascus wall. × 665. FIGS. 9–10. Young asci showing variation in shape. × 665. FIG. 11. Young perithecia. × 25. FIG. 12. Perithecium before extrusion of ascospores. × 25. FIGS. 13–14. Perithecia showing development of more than one neck. × 25. FIGS. 15–16. Mature perithecia showing typical cirri of ascospores. × 25.

Lophotrichus martinii. FIGS. 17–33

FIG. 17. Mature perithecium with cirrus of ascospores not shown. × 65. FIG. 18. Terminal hair showing thick wall and curved tip. × 500. FIG. 19. Portion of terminal hair showing development of short branches. × 500. FIG. 20. Portion of terminal hair showing dichotomous branching. × 500. FIG. 21. Portion of terminal hair showing encrustations. × 335. FIG. 22. Lateral hair. × 500. FIG. 23. Mature ascospores, in outline, showing thin end walls. × 835. FIG. 24. Germinating ascospore, in outline. × 835. FIG. 25. Outline of mature ascus just prior to breakdown of ascus wall. × 665. FIGS. 26–27. Young asci showing variation in shape. × 665. FIG. 28. Young perithecia. × 25. FIG. 29. Young perithecium before extrusion of ascospores. × 25. FIGS. 30–31. Perithecia showing development of more than one neck. × 25. FIGS. 32–33. Mature perithecia showing typical cirri of ascospores. × 25.

* Dotted line with figures of perithecia indicates level of substrate.

NOTES AND BRIEF ARTICLES

TARGET SPOT OF COWPEA AND SOYBEAN. Recently Olive *et al.* (Phytopath. **35**: 822–831. 1945) described what appeared to be a new leaf-spot disease of cowpea and soybean. The causal organism was named *Helminthosporium vignae*. However, an earlier paper describing the disease and its causal agent has just come to the writer's attention. Kawamura (Fung. Nippon Fungological Soc. **1**: 14–20. 1931) found the fungus on cowpea in Japan and described it as *Cercospora vignicola* nov. sp. During the past year, Liu (Bot. Bull. Acad. Sinica **2**: 69–80. 1948) reported that the disease is a common one in China on cowpea and soybeans. He found that leaves and pods of the soybean are attacked by the fungus, and that the fungus may penetrate into the seeds and spot them or even prevent them from maturing. Liu prefers Kawamura's classification of the pathogen as a *Cercospora*.

The present writer remains of the opinion that this fungus should be classified as a species of *Helminthosporium* rather than *Cercospora*. It is true that if diseased leaves of cowpea and soybean are placed in a moist chamber most of the conidia which are produced will be long and narrow. But in nature the conidia are usually broader, typically less than ten times as long as broad. At maturity they are quite brown in color, and their walls and septa become characteristically thickened. Thus the fungus appears to fit better into the *Helminthosporium* concept. In view of these considerations, the writer proposes the following classification of the fungus: **Helminthosporium vignicola** (Kawamura) comb. nov. (Syn. *Cercospora vignicola* Kawamura; *Helminthosporium vignae* Olive).—Lindsay S. Olive, Louisiana State University.

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Zahlbruckner, A. Catalogus lichenum universalis. (10 vols.) $175.00

The first and second of these works are the points of departure for the nomenclature of all groups of fungi except the myxomycetes. The third lists the literature of mycology and lichenology, and on fungus diseases, to 1910, and is of great service in running down references to work in these fields. The fourth is the lichenologists' equivalent of Saccardo. For years all have been for the majority of workers impossible to secure.

These works will be published only if the number of sales will be great enough to support the project. It is therefore requested that *tentative* subscriptions, either personal or institutional, be sent to the undersigned.—D. P. Rogers, New York Botanical Garden, New York 58, N. Y.

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